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ELISHA MITCHELL Scientific Society

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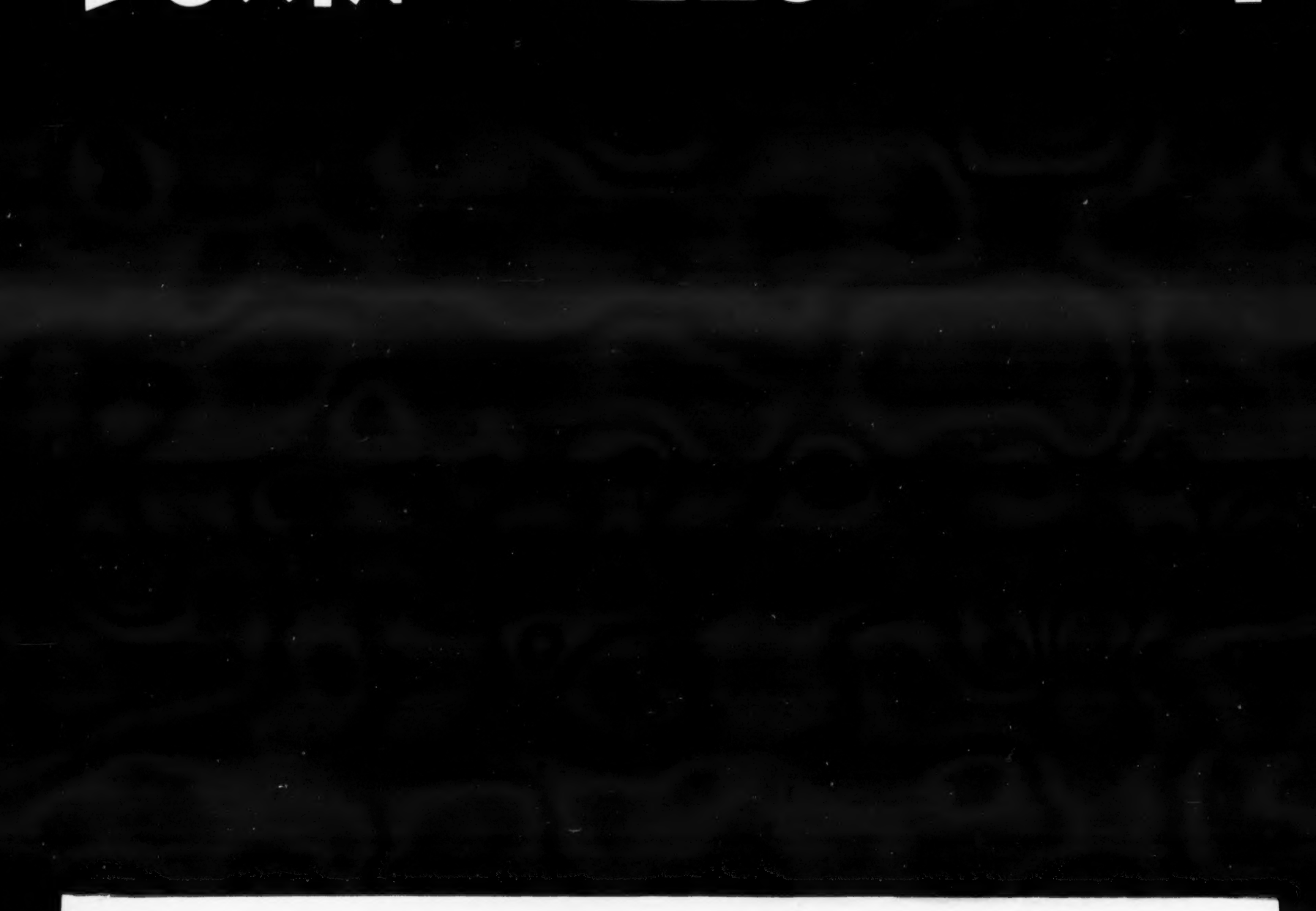


JOURNAL

OF THE

Elisha Mitchell Scientific Society

Volume 63 December 1947 No. 2

PROCEEDINGS OF THE FORTY-FOURTH ANNUAL MEETING OF THE NORTH CAROLINA ACADEMY OF SCIENCE

WAKE FOREST COLLEGE, WAKE FOREST, N. C.

The forty-fourth annual meeting of the North Carolina Academy of Science was held at Wake Forest College on May 9 and 10, 1947.

The Academy convened in a general session at 10:30 a.m. on the first day with President John N. Couch presiding. After the presentation of five papers the session adjourned for lunch at noon.

The second general session was called to order at 2:00 p.m. the same day. After the reading of seven papers the session closed and was followed by the business meeting.

The minutes of the 1946 meeting were approved as published in the Journal of the Elisha Mitchell Scientific Society **62**: 109–143. The reports of the various committees were then presented as follows.

REPORT OF THE EXECUTIVE COMMITTEE

The Executive Committee met in Durham, N. C., on May 8, 1947, and at at Wake Forest on May 9, 1947. At these meetings the following matters were transacted:

1. It was recommended that the members of the High School and Conservation Committees be appointed for two years and that the membership be staggered.
2. A motion to pay the Elisha Mitchell Scientific Society $600.00 for the years 1944 and 1947 was passed. If necessary the funds of the Savings Account are to be used for this purpose.
3. The Treasurer was authorized to pay all outstanding bills for operating expenses.
4. The committee authorized the Treasurer to submit his financial report as of July 1, 1947, to the auditing committee.
5. The following were elected to membership in the Academy:

Adams, Carl L., Eastern Carolina Teachers College
Arbuckle, Wendle Sherwood, N. C. State College

Ashworth, Ralph Page, Mars Hill College
Bailey, Joseph Randle, Duke University
Ball, Ernest, N. C. State College
Barney, Charles Wesley, Duke University
Bell, Thomas Alexander, U. S. Department of Agriculture, Raleigh
Berkut, Michael K., N. C. State College
Blalock, Thomas Jacks, N. C. State College
Bowman, Frank Otto, Jr., University of North Carolina
Bunn, Charles Ivy, N. C. State College
Conner, James Thomas, N. C. State College
Danieley, James Earl, Elon College
Dawley, Charlotte Webster, Woman's College U. N. C.
Derrick, Jasper Otto, Eastern Carolina Teachers College
Fox, William Basil, N. C. State College
Frey, David G., University of North Carolina
Gay, Roland Lafayette, Wake Forest College
Greene, George William, Catawba College
Grosch, Daniel Swartwood, N. C. State College
Harrington, John Wilbur, University of North Carolina
Harris, Mrs. D. P., N. C. State College
Hill, Benjamin Harvey, High Point College
Hoke, Roy Edward, Psychological Service Center, Charlotte
Ingram, William Prentiss, Jr., N. C. State College
Jensen, James H., N. C. State College
Jones, Claiborne Stribling, University of North Carolina
Jones, Harold Charles, Eastern Carolina Teachers College
Jones, Hubert A., Wake Forest College
Kelley, Douglas McGlashan, Bowman Gray School of Medicine
Kelly, Joseph Wheeler, N. C. State College
Kozlowski, Theodore Thomas, Duke University
Lemlich, Benjamin Rothbell, N. C. State College
Levadie, Benjamin Theodore, N. C. State College
Longest, Mrs. Pauline Moser, University of North Carolina
McEwen, Mildred Morse, Queens College
Maynard, Poole, Atlantic Coast Line R. R., Atlanta, Georgia
Milner, Mrs. Clyde A., Guilford College
Morris, Ailene, University of North Carolina
Mundorff, Maurice J., U. S. Geological Survey, Raleigh
Ohmer, Harvey B., N. C. State College
Oliver, George M., N. C. State College
Parker, Herman Manley, Wake Forest College
Pietenpal, Clarence John, Davidson College
Price, Walter Eugene, N. C. State College
Richenberg-Hackett, Wally, Duke University
Scofield, Herbert Temple, N. C. State College
Shields, James Duncan, Durham High School
Smith, Juanita Maxine, N. C. State College
Walldroff, Mrs. Bessie, Elon College
Warren, John B., Duke University
Weaver, Richard Lee, North Carolina Resource-Use Education Commission, Chapel Hill
Wilson, William, N. C. State College

6. Twenty former members were reinstated.

7. The Secretary reported the following losses of members:

1. By resignation, 2.
2. By non-payment of dues, 25.
3. By death, C. S. Brimley, N. C. State Museum.

8. The committee accepted the invitation of Davidson College to meet there in 1948.

The above reports of the Executive Committee were adopted.

A proposal by the Executive Committee to offer the Poteat Award to only one section annually was not approved by the Academy. A motion by Dr. Ruth Addoms that the award be given for a meritorious paper was passed. The Academy approved a motion making it mandatory for members to submit, at least 30 days before the annual meeting, abstracts of their papers to the Secretary in order to be eligible for the Poteat Award.

TREASURER'S REPORT

Financial Statement as of July 1, 1947

Receipts	
Bank Balance (checking acc't from H. L. Blomquist) Oct. 1, 1946	$46.26
Sale of file cabinet	3.00
Gifts	
J. D. Ives	10.00
Ornithological Essay Prize, 1947	20.00
A. A. A. S. Research Grant	100.00
Initiation fees and dues	584.00
From Savings Account	216.53
Total	979.79
Expenditures	
Stamps, stamped envelopes, postals	60.10
Stationery (N. C. State College Print Shop)	15.76
Office Supplies	8.14
Express charges	.72
Secretarial Commission (10% of dues)	58.00
Expenses to meeting	2.00
Secretarial Aid	52.00
Bank Charge	.84
Programs (N. C. State Print Shop)	20.23
Academy Award	20.00
Ornithological Essay Award	20.00
Transferred to New Savings Account	66.53
Elisha Mitchell Scientific Society (1944 and 1947)	600.00
Botany Department, Duke University (for reprints of proceedings)	10.00
Total	934.37
Bank Balance	45.42
	979.79

Savings Account

Balance Oct. 1, 1946	215.76
Interest, June 23, 1947	1.07
	216.83
Tax	.30
Transferred to checking account for payment to the Elisha Mitchell Scientific Society	150.00
Balance, July 1, 1947	66.53

Total Assets

Checking Account	45.42
Savings Account	66.53
U. S. Savings Bonds (Maturity Value)	300.00
Total	411.95

REPORT OF THE AUDITING COMMITTEE

On the thirtieth day of June, 1947, we, the undersigned, have examined this account and found it to be correct.

(Signed) MARY E. YARBROUGH,
FRANK W. SHERWOOD,
C. H. BOSTIAN, *Chairman.*

REPORT ON ACADEMY CONFERENCE AT THE BOSTON MEETING OF THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE (1946)

There were 28 delegates present representing 24 Academies of Science.

Dr. Otis Caldwell, General Secretary of A. A. A. S., spoke of the great responsibility borne by the affiliated Academies for improvement of services and relations to state educational and industrial enterprises, the preparation of scientific personnel, the sponsoring of Science Clubs and development of the Junior Academy as a valuable educational procedure and channels for directing interested and competent young people into the field of science.

This led to consideration by the Academy of the agenda prepared by Chairman E. F. Degering as follows:

1. In what ways may a State Academy improve its services and relations with its own state educational and industrial enterprises?

Dr. G. O. Carter suggested that the Academies could make substantial contributions to education and industry by sponsoring lecture programs on human relationships throughout the year. Professor H. D. Carle of New Hampshire mentioned that their Academy-sponsored mountain experiment station has obtained cooperation and financial aid from industry and the Federal Government in its development.

Some State Academies have made valuable industrial contacts. It was suggested that State Academies have committees who have the duties of contacting industrial concerns for research aid. Also that industrialists be invited to take part in the Academy meetings.

The sponsoring of research in the smaller institutions was suggested as a responsibility of the Academies. The A. A. A. S. research grants might be directed to the smaller institutions.

2. What can be done by the State Academy toward developing valuable additional scientific personnel?

Dr. H. A. Webb of the Tennessee Academy, spoke of the importance of continuing and developing the Science Talent Search. Tennessee has in addition developed a State Science Talent Search. This may be of importance in guiding the most talented young people into the field of science.

It was suggested that something be done for the junior scientists through industry. Industry does not know about these needs. Closer cooperation of the Academies with industry is very desirable.

Dr. E. C. L. Miller of the Virginia Academy presented a paper on the responsibilities of the State Academy. We quote one paragraph dealing with this matter, "Methods of encouraging young scientists, as well as discovering future scientists, include the development of science clubs in the high schools, encouraging young people to participate in science talent searches, and making possible the basic education of those who show promise. No potential scientist must be allowed to drift into a field for which he is less suited for want of proper guidance, nor be permitted to enter science without the requisite basic training. Our very survival as a nation may depend on how well we do this."

There are 10,500 Science Clubs in the United States. Representatives from the 52 clubs in Indiana meet with the Academy of Science at their regular autumn meeting. There are 151 Science Clubs in South Carolina affiliated with the S. C. Academy of Science.

3. How may the service of the Junior Academy be increased?

After considerable discussion the conference passed a motion requesting the officers and directors of the A. A. A. S. to consider possible measures of working through the Academies of Science for the encouragement of Junior Academies or Junior Science activities, and prepare a report for publication on the present status of Junior Academies of Science in the United States, with recommendations.

4. Should the State Academies supplement their research funds? If so, how and for what specific purposes?

Dr. O. W. Caldwell pointed out that in order to obtain research funds from the A. A. A. S. application must be made by each Academy for these funds. Such funds cannot be used for publication purposes. As to supplementing these grants from the A. A. A. S., he suggested that a resolution might be made to state some kind of principle of obtaining ways of setting up and underwriting funds for the sole use of the Academy of Science for research.

A. S. PEARSE.

The above report was accepted by the Academy.

REPORT OF THE LIFE MEMBERSHIP COMMITTEE

The committee, consisting of G. C. Mackie, G. H. Satterfield, O. C. Bradbury, and F. G. Hall, Chairman, nominated the following scientists for election to life

membership in recognition of their outstanding contributions and service to the field of science:

J. G. Boomhour, retired Dean of Meredith College
R. E. Coker, Zoology Department, U. N. C.
J. B. Derieux, retired Professor of Physics, N. C. State.
C. M. Heck, retired Head of Physics Department, N. C. State
A. S. Pearse, Zoology Department, Duke University.

The Academy unanimously elected these scientists to life membership.

REPORT OF THE RESOLUTIONS COMMITTEE

Be it resolved that we, the members of the North Carolina Academy of Science, do hereby gratefully acknowledge the hospitality and kindness of the officers of administration and faculty of Wake Forest College in extending the use of the college buildings and their facilities at the Academy's forty-fourth annual meeting.

Be it also resolved that we thank the Committee on arrangements, O. C. Bradbury, Chairman, C. S. Black, H. A. Jones, A. C. Reid, W. E. Speas, B. E. Smith, and E. C. Cocke, which has worked most efficiently to make us enjoy our visit through the excellent facilities provided for the meeting, the complimentary dinner, and the general good fellowship.

Be it further resolved that a copy of these resolutions become a part of the minutes of the Academy's meetings, and that a copy be sent to Dean D. B. Bryan and to President T. D. Kitchin of Wake Forest College.

OTTO STUHLMAN, JR.

The resolution was adopted.

REPORT OF THE RESEARCH GRANTS COMMITTEE

There were no applications for the A. A. A. S. Research Grant prior to the annual meeting of the Academy. The committee, now consisting of W. E. Speas, O. C. Bradbury, G. R. MacCarthy, and B. W. Wells, received two applications for the 1947 grant. After due consideration by correspondence it was decided to divide the grant equally between Dr. E. C. Cocke of Wake Forest College and Mr. T. L. Quay of N. C. State College. Each received the sum of $64.25. Dr. Cocke is to use the funds for expenses incurred in a study of the Myxophyceae of North Carolina and Mr. Quay for his study on the ecology of the Savannah Sparrow.

E. W. BERRY,
W. E. SPEAS,
O. C. BRADBURY,
J. P. GIVLER, *Chairman.*

REPORT OF THE POTEAT AWARD COMMITTEE

The Committee held a meeting at noon on Saturday and discussed at length the difficult problem of selecting a meritorious paper presented this year. After

due consideration it was decided to grant the award to Dr. Otto Stuhlman, Jr., for his excellent paper, "A dynamical analysis of the movements of the Venus flytrap."

I. E. GRAY
J. W. LASLEY, JR.,
G. H. SATTERFIELD,
A. D. SHAFTESBURY,
J. L. STUCKEY,
P. J. KRAMER, *Chairman.*

REPORT OF THE HIGH SCHOOL SCIENCE COMMITTEE

Dr. A. D. Shaftesbury, Chairman of the Committee, gave an informal report of plans for the coming year. It is hoped that arrangements can be made for regional fairs for the exhibition of projects. The top two projects of these fairs would be exhibited at the annual meeting of the Academy. Announcement was made of the following winners of the awards and prizes:

THE ACADEMY AWARD

The award for the High School Science Project was presented to the Tenth Grade Biology Class, Glenwood High School, Lemon Springs, Miss Mary Thomas Carswell, Sponsor.

BIOLOGICAL EXHIBITS AWARD

(Carolina Biological Supply Company)

First prize: Tenth Grade, Rock Ridge High School, Mrs. Will Rhodes, Sponsor.

ORNITHOLOGICAL ESSAY

First prize: James Stamey, High School Department, Warren Wilson Junior College, Swannanoa.
Second prize: Bawlant Anand Paradkar, High School Department, Warren Wilson Junior College, Swannanoa.
Third prize: John T. Butters, Jr., New Hanover High School, Wilmington.

1946 FORESTRY ESSAY AWARD

First prize: Sallie Massey, Hugh Morson High School, Raleigh.
Second prize: Lois Templeton, Union Grove High School, Union Grove.
Third prize: Calista Weisner, Union Grove High School, Union Grove.

CONSERVATION COMMITTEE

The Committee made no formal report. The following letter was written to the President of the Academy by J. S. Holmes, Chairman, and accepted by the Academy. "Since your chairman retired from public service a year ago and is at the present time recovering from a serious illness, he does not feel in any way

qualified to prepare a report so he is submitting this very brief sketch of the Conservation field, touching only some high spots.

"Use without waste is about the only conservation practice that can be applied to our non-renewable resources such as oil, coal, minerals and even our soils. But the renewable resources, forests, wildlife, wild flowers, fresh and salt water life, must be used so that the natural balance be restored and maintained.

"There are now perhaps some fifty trained foresters employed by the public to help landowners cut their timber so that the productivity of the forest may be maintained, yet on by far the larger number of timber operations little or no attention is paid to the future possibilities of the stand; so many landowners are unwilling to leave growing sufficient salable trees to insure a profitable second crop.

"When it comes to wildlife, which may or may not include insectivorous and song birds, and other non-game birds and animals, I am a layman, but I fail to see how a group of sportsmen are likely to represent adequately the varied interest of the public in such resources.

"And what about our fish and other inhabitants of our salt and fresh waters? We know little of the habits even of our 'sea-food' species, but we do know that the fishermen catch and the markets supply smaller and smaller specimens year by year. This sooner or later leads to extinction.

"The fields for scientific study and the practical application of our knowledge of our renewable natural resources are both wide open and pleading for unselfish and public-spirited attention."

The following memorial report was presented at the meeting.

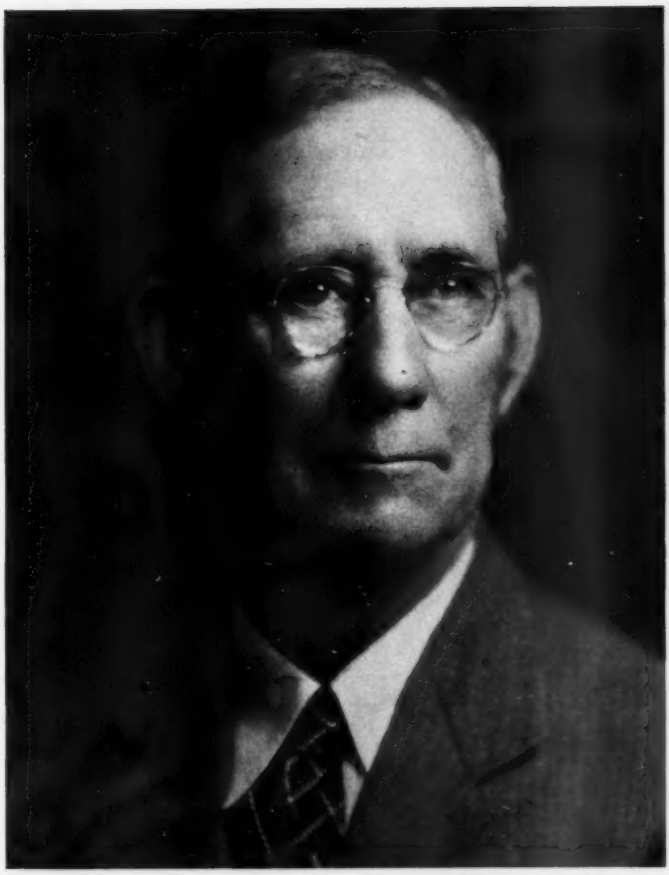
CLEMENT SAMUEL BRIMLEY

Clement Samuel Brimley, Associate Entomologist with the North Carolina Department of Agriculture, died as he was preparing to go to his office on July 23, 1946.

Dr. Brimley was born at Great Linford, Buckingham County, England, on December 18, 1863. He later lived on a large farm in the village of Willington, 4 miles west of Bedford. Formal education was confined to seven or eight terms at the Bedford County School.

Late in 1880 farming efforts were stalemated and he, his father, mother, two brothers and two sisters determined to leave England for Canada or Australia. Chance brought them in contact with an Immigration Agent of the North Carolina Department of Agriculture and they secured through tickets to Raleigh by way of New York and Norfolk, arriving on the last day of 1880. The night was spent in a hotel that later became the building in which he and his brother, H. H. Brimley, achieved scientific distinction.

About 1881 these brothers began collecting natural history specimens in what was an unworked field. Their basic text was a small book, "Taxidermy Without a Teacher." H. H. Brimley went into the building of a State Museum and C. S. Brimley continued the supply business. With scant formal education, the



CLEMENT SAMUEL BRIMLEY
1863–1946

latter was primarily a task of laborious research in biology. He built his own keys and made his identifications the hard way. In 1919 he joined the Entomology Division of the N. C. Department of Agriculture and scientific workers were already beating a path to his door. He was contributing many original scientific articles in the whole field of zoology that brought him recognition and admiration. Additional recognition came when the faculty of the University of North Carolina petitioned in 1938 that he be awarded an honorary doctorate. This was done at the June commencement. In 1930 the U. S. Biological Survey awarded him a 46-year certificate for bird migration studies.

Among his major contributions was the co-authorship of the volume, "Birds of North Carolina" in 1919 and 1942. His volume, "Insects of North Carolina," came out in 1938. Between 1940 and 1946 he had published "Amphibians and Reptiles of North Carolina," "Mammals of North Carolina" and, as co-author, "Poisonous Snakes of the Eastern United States." The latter has, in two years, been distributed to the number of 25,000 copies.

For more than 60 years, with infinite patience and perseverance, he had kept definite records, from personal observation and correspondents, of all forms of plant and animal life of his adopted State, plus related forms from without. His series of skins and preserved specimens are a treasury that has been open to research workers. His insect collection for his State numbers more than a half million, of more than 7,000 species. Not the least of his accomplishments was the inspiration and material help and guidance he gave youth. Neighboring children sat entranced at his feet to hear his stories of wild creatures. Boys, girls, and adults shared the privilege of hiking afield with him. He was a generous citizen of his community and was endeared to members of his Church and Fraternal Organization.

His scientific associations were kept alive by many memberships. He was a founder and honorary member of the N. C. Academy of Science, the N. C. Bird Club, the Raleigh Bird Club and the Raleigh Natural History Club.

HARRY T. DAVIS.

The above report was adopted with a rising vote.

REPORT OF THE NOMINATING COMMITTEE

The Committee submitted the following nominations:

President: F. G. Hall, Duke.

Vice-president: A. D. Shaftesbury, Woman's College U. N. C.

Secretary-Treasurer (2 years): Reinard Harkema, N. C. State.

Member of the Executive Committee: I. E. Gray, Duke. John N. Couch, U. N. C., was appointed by the Executive Committee to replace F. G. Hall for one year.

Member Research Grants Committee: G. R. MacCarthy, U. N. C., and B. W. Wells, N. C. State.

There being no nominations from the floor, the Secretary was asked to cast the ballot and declare the nominees elected. This concluded the business and the meeting was adjourned.

At 7:00 p.m. the Academy members enjoyed a complimentary dinner given by Wake Forest College. The Vice-president Dr. Ruth M. Addoms presided at the evening meeting. She introduced Dean D. B. Bryan of Wake Forest College who welcomed the Academy. This was followed by the Presidential Address by Dr. John N. Couch who spoke on "Symbiosis in Animals and Plants."

All of the sectional meetings were held on Saturday morning. These were well attended. The following officers were elected for the respective sections:

Biochemistry and Physiology: Chairman, F. G. Hall; Secretary, Mary E. Yarbrough.

Botany: Chairman, B. W. Wells; Secretary, Lewis E. Anderson.

Geology: Chairman, T. G. Murdock; Secretary, E. W. Berry.

Mathematics: Chairman, V. A. Hoyle; Secretary, F. G. Dressel.

Physics: No meeting.

Psychology and Clinical Psychology: Chairman, D. J. Moffie; Secretary, William McGehee.

Wildlife: Chairman, Harry T. Davis; Secretary, Willis King.

Zoology: Chairman, W. O. Puckett; Secretary, G. W. Wharton.

The following constitute the personnel of the standing committees:

Executive: F. G. Hall, A. D. Shaftesbury, Reinard Harkema, E. H. Hall, J. N. Couch, I. E. Gray.

Research Grants: W. E. Speas (1948), O. C. Bradbury (1949), G. R. MacCarthy (1950), B. W. Wells (1950).

Dr. F. G. Hall, President of the Academy for 1948, announces the appointment of the following committees:

Auditing: C. H. Bostian, Mary E. Yarbrough, F. W. Sherwood.

Conservation: C. F. Korstian, J. S. Holmes, Arthur Stupka.

High School: A. D. Shaftesbury, Ruby Williams, J. H. Highsmith, J. S. Meares, E. E. Brown.

Legislative: Z. P. Metcalf, B. C. Burgess, H. F. Prytherch, B. W. Wells.

Life Membership: O. C. Bradbury, G. C. Mackie, G. H. Satterfield, J. H. Ferguson.

Nominating: J. N. Couch, M. L. Braun, H. L. Blomquist.

Poteat Award: Mary E. Yarbrough, L. E. Anderson, E. W. Berry, F. G. Dressel, Reinard Harkema, Willis King, William McGehee, G. W. Wharton.

Resolutions: H. R. Totten.

Resource Use: R. L. Weaver, H. J. Oosting.

Representative on A. A. A. S. Council: D. B. Anderson; alternate, J. N. Couch.

Representative to Academy Conference: M. L. Braun.

Secretarial and Constitutional: H. R. Totten, H. L. Blomquist, Reinard Harkema.

Approximately 250 members and guests registered during the meeting. The following papers were presented during the meeting. Those marked with

an x are abstracted in these proceedings; those marked with a † are published in full.

GENERAL SESSIONS

Address of Welcome. D. B. BRYAN, Dean, Wake Forest College.

Presidential Address: Symbiosis in animals and plants. JOHN N. COUCH, President of the Academy.

Carolina Bays 1947. W. F. PROUTY, U. N. C.

A bay in a bay. B. W. WELLS, N. C. State.

A dynamical analysis of the movements of the Venus flytrap. OTTO STUHLMAN, JR., U. N. C.

High speed motion pictures as a tool in research. W. O. PUCKETT, Davidson.

The proposed Institute of Biological Sciences. P. J. KRAMER, Duke.

Prime numbers—an antidote to mental diminution. G. W. GREENE, Catawba.

Natural history of the Great Smokies. ARTHUR STUPKA, Great Smoky Mountains National Park.

A comparison of virgin spruce-fir forest in the northern and southern Appalachians. H. J. OOSTING and W. D. BILLINGS, Duke.

Ecological problems in Albemarle Sound. H. F. PRYTHERCH, U. S. Fisheries Laboratory.

Fisheries of the Philippines. D. G. FREY, U. N. C.

x*Methods of cultivation in relation to crop yield.* K. J. SHAW and T. E. SMITH, McNair Seed Co.

Resource-use Education in North Carolina. R. L. WEAVER, N. C. Resource-Use Education Commission.

BIOCHEMISTRY AND PHYSIOLOGY SECTION

x*Proteolytic enzymes and blood coagulation.* J. H. FERGUSON, B. L. TRAVIS, and E. B. GERHEIM, U. N. C. School of Medicine.

x*Activation and inhibition of plasma tryptase.* B. L. TRAVIS, J. H. FERGUSON, and E. B. GERHEIM, U. N. C. School of Medicine.

x*Action of coagulase with plasma constituents.* E. B. GERHEIM, J. H. FERGUSON, and B. L. TRAVIS, U. N. C. School of Medicine.

Action of ethanolamine and ethanolamine derivatives on lipide phosphorylation. CAMILLO ARTOM and W. E. CORNATZER, Bowman Gray School of Medicine.

x*The gastro-intestinal absorption and distribution of isopropyl alcohol in dogs.* JOAN WAX and A. J. LEHMAN, U. N. C. School of Medicine.

x*Renal hyperemia in dogs induced by oral administration of cinchona alkaloids.* E. P. HIATT and V. SUHRIE, U. N. C. School of Medicine.

x*The excretion of the blue dye T-1824 in the bile.* A. T. MILLER, U. N. C. School of Medicine.

x*Nicotinic acid content of goat's milk.* LYNN MADDRY, State Laboratory of Hygiene, and G. H. SATTERFIELD, N. C. State.

BOTANY SECTION

A new species of Salix. B. E. SMITH, Wake Forest.

The use of polyvinyl alcohol as a microscopic mounting medium for algae. L. G. WILLIAMS, Duke.

†*A study of some algal pigments.* L. A. WHITFORD, N. C. State.

Preliminary observations on marine denitrifying bacteria of North Carolina coastal waters. H. J. HUMM and H. G. LOFTIN, Duke Marine Laboratory.

x*Light and water in growth and composition of piedmont forest tree species.* T. T. KOZLOWSKI, Duke.

x*Effects of fertilizer on growth rates of Ilex lucida.* MARY CAUGHEY, E. C. T. C.

Asplenium monanthes L. in South Carolina. H. L. BLOMQUIST, Duke (Read by title only)

The European larch, Larix decidua, the Oriental hydrangea, Hydrangea paniculata, and Spiraea Bumalda var. Anthony Waterer as escapes. H. R. TOTTEN, U. N. C.

x*North Carolina Gelidium agars.* O. CHRISTINE WILTON, E. C. T. C., and H. J. HUMM, Duke Marine Laboratory.

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x*Principles of wild turkey management.* ROBERT WHEELER, N. C. Department of Conservation.

The National Park Service's wildlife program. ARTHUR STUPKA, Great Smoky Mountains National Park.

An investigation of North Carolina's coastal fisheries. NELSON MARSHALL, U. N. C.

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Temperature gradients off Hatteras and their influence on fish distribution. NELSON MARSHALL, U. N. C.

x*The relation between gill surface and activity in marine fishes.* I. E. GRAY, Duke.

x*The correlation between animal size and appendage size in Habrobracon and its use in genetic investigation.* D. S. GROSCH, N. C. State.

†*Breeding habits of cave spiders.* J. D. IVES, Carson-Newman

Whence and where taxonomy? Z. P. METCALF, N. C. State.

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x*Ontogenetic variation in certain taxonomic characters in the dusky salamander, Desmognathus f. fuscus.* J. R. BAILEY, Duke.

NORTH CAROLINA SECTION OF THE AMERICAN CHEMICAL SOCIETY

Studies in liquid sulfur dioxide. I. Sulfonation of benzene. LEE LEISERSON and R. W. BOST, U. N. C.

The problem of the Periodic System. O. H. BROWNE, Lenoir Rhyne.

Isolation and evaluation of sources of variation in the serum calcium method. J. A. WAYBREW, G. MATRONE, and HARTLEE M. BAXLEY, N. C. State.

Volatilization of the elements with acid mixtures. S. Y. TYREE and G. G. MARVIN, U. N. C.

Chloro-bis-(trifluro-methyl)-benzenes. C. K. BRADSHER, P. M. BOST, M. E. HOBBS, J. H. SAYLOR, P. TARRANT, and E. C. WEST, Duke.

The preparation of heterocyclic fluorine compounds using the Scheimann reaction. ARTHUR ROE and G. F. HAWKINS, U. N. C.

Ignition temperatures of rigid plastics. E. M. SCHOENBORN and D. S. WEAVER, JR., N. C. State.

Abstracts:

Methods of Cultivation in Relation to Crop Yield. K. J. SHAW and T. E. SMITH.

Plowman's Folly, by Faulkner, raised the question of the most effective procedure in soil preparation and cultivation after the growth of a winter cover crop. Corn, cotton, and tobacco were used as test plants in 1944 through 1946 inclusive on Norfolk sandy loam, deep phase. Corn and cotton were preceded by Austrian winter peas and tobacco by rye. Formula and rate of fertilization was standard for all plots of each crop. Two cultural procedures were used. First, the winter crop was disked into the soil surface and the crops planted in a shallow furrow and cultivated flat. Second, the winter crop was turned into the soil with a mold-board plow, disked and the crops planted and cultivated in the usual way: corn was seeded in a furrow and cultivated flat; cotton was seeded on a list and cultivated on a low ridge; tobacco was transplanted on a list and cultivated according to the ridge method. The plots were 0.1 acre in size. Two replications of the two procedures on each crop were grown each year. Average yields for the 3-year period on the crops grown in procedures A and B were respectively: Corn, 3,087 and 3,185 pounds per acre on cob; cotton, 614 and 679 pounds per acre of list; tobacco, 1,523 and 1,541 pounds per acre of cured leaf. The results showed that plowing combined with the usual procedure of planting and cultivation gave slightly higher yields.

Proteolytic enzymes and blood-coagulation. J. H. FERGUSON, B. L. TRAVIS, E. B. GERHEIM.

Natural Tryptase enzyme, commonly persisting as an impurity in plasma or tissue protein products, has a thromboplastic action, in the sense of aiding calcium and cephalin (or thromboplastic phospholipid) to activate prothrombin in the first-phase of the blood-clotting mechanism. Like the ordinary proteolytic effects (including fibrinogenolysis, fibrinolysis, prothrombinolysis, and

thrombinolysis) the thromboplastic action of natural tryptase is antagonized by known trypsin-inhibitors. Soybean protein (but not the pancreatic polypeptide) has, in addition, a more direct antithromboplastic (anticephalin) effect, somewhat like heparin (or antiprothrombin). Heparin is not antitryptic in physioligical concentrations and its antithrombic action cannot use the soybean protein as a co-factor. None of the agents cited (except possibly heparin) have any significant effects on the thrombin-fibrinogen interaction.

The physiological explanation of the dependence of the thromboplastic tryptase action upon adequate available calcium and especially phospholipid, is fundamental both to (1) the mode of enzyme action, and (2) the basically non-enzymic mechanism of thrombin formation. These experiments point the way to a new field of clinical possibilities, which we are currently exploring, with regard to the role of tryptase enzymes in normal and pathological variations of the blood clotting system. (This work has been aided by a grant from the John and Mary R. Markle Foundation.)

Activation and Inhibition of Plasma Tryptase. B. L. TRAVIS, J. H. FERGUSON, and E. B. GERHEIM.

Timing of the disappearance of clotting capacity of fibrinogen, as this protein is digested by the proteolytic enzyme, is used to demonstrate the presence of natural plasma *tryptase*. This protease exists mainly in an inactive precursor form (tryptogen) in certain fibrinogen-rich plasma protein fractions of human, but not bovine, origin. The value of *streptokinase* ("streptococcal fibrinolysin") for activation of the enzyme-precursor is confirmed, and tests are presented to show that the failure to secure proteolytic effects in the bovine plasma fractions is due to lack of tryptogen and not to tryptase-inhibitors.

Antitryptase effects of (a) crystalline inhibitors, from pancreas and soybean, and (b) crude and fractionated egg-albumen, favor the characterization of the plasma protease as a "tryptase". (This work has been aided by a grant from the John and Mary R. Markle Foundation.)

Action of Coagulase with Plasma Constituents. E. B. GERHEIM, J. H. FERGUSON, and B. L. TRAVIS.

1. A brief historical review of the coagulase phenomenon is discussed along with the method of determining coagulase positive *Staph. aureus* for tests in this series of experiments.

2. Coagulase action under various conditions on plasma, action on fibrinogen, role in prothrombin activation and results with other plasma constituents are discussed. (This work has been aided by a grant from the John and Mary R. Markle Foundation.)

The Gastro-intestinal Absorption and Distribution of Isopropyl Alcohol in Dogs. JOAN WAX and A. J. LEHMAN.

An investigation was made of the gastro-intestinal absorption of isopropyl alcohol and its distribution in the tissues. Absorption was found to be highest in

the jejunal portion of the intestine. Absorption was not significantly affected by variations in concentration or area of absorptive surface, although tissue distribution increased considerably with increasing area of absorption. The greatest part of the absorption occurred within the first half hour and was 99 per cent complete after two hours. The presence of absorbed isopropyl alcohol did not significantly affect the absorption of successive quantities of isopropyl alcohol, but ethyl alcohol exhibited a systemic inhibition on the intestinal absorption of isopropyl alcohol.

There was no observable constancy in the tissue-blood alcohol concentrations. Brain alcohol concentration was somewhat higher than any of the other tissue alcohol concentrations except in one series of experiments; while muscle alcohol, with one exception, was the lowest. (This research was supported by a grant from the Standard Alcohol Company of New York, through the courtesy of Mr. James Park.)

Renal Hyperemia in Dogs Induced by Oral Administration of Cinchona Alkaloids. E. P. HIATT and VIRGINIA SUHRIE.

1. The effect of oral doses of the sulfates of the cinchona alkaloids on the effective renal plasma flow, as measured by p-aminohippurate clearances, and the glomerular filtration rate, as measured by creatinine clearance, has been studied in dogs.

2. These agents cause an increase in effective renal plasma flow of 30 to 50 per cent beginning within 30 minutes and lasting for several hours, according to the sojourn of the drug in the blood.

3. The filtration rate is also increased but to a smaller degree.

4. There is little change in blood pressure but there is usually some acceleration of the pulse rate.

5. The manner of action and possible therapeutic applications are discussed. (This research was supported by a grant from the Samuel S. Fels Fund.)

The Excretion of the Blue Dye T-1824 in the Bile. A. T. MILLER, JR.

The hepatic removal of intravenously injected T-1824 has been studied because of its bearing on the interpretation of the T-1824 disappearance curve. Spectrophotometric analyses of hepatic bile and of blood serum, supplemented by chemical separation of the dye from bile, permitted the following conclusions:

(1) Very little dye appears in hepatic bile in the first 30 minutes after injection.

(2) Maximum concentration in bile is reached 60 to 90 minutes after injection; dye concentration then declines slowly, paralleling the fall in plasma dye concentration.

(3) Dye concentration in the bile is always lower than (usually less than one-half) the simultaneous plasma dye concentration, but roughly proportional to it.

(4) The equilibrium dye concentration in bile is not due to the saturation of a secretory mechanism (as in renal Tm) because a second injection of dye results in a second rise in dye concentration in bile.

(5) The concentration of dye in bile bears no obvious relation to the rate of bile flow within the limits of flow rate encountered (2 to 10 ml. of bile per hour).
(6) The excretion of dye in the bile in the first 2 to 5 hours following injection accounts for only 2 to 5 per cent of the dye which leaves the bloodstream in that interval. Hence liver function is presumably not a limiting factor in the rate of dye disappearance.
(7) The concentration of dye in bile parallels (but at a lower level) that of dye in thoracic duct lymph.

Riboflavin and Nicotinic Acid Content of Goat's Milk. L. G. MADDRY and G. H. SATTERFIELD.

Various workers have analyzed goat's milk for riboflavin and nicotinic acid. The reports varied through a wide range. Due to this variation of results it was thought advisable to make additional studies on the amount of these two vitamins in goat's milk. Two hundred and thirty samples of goat's milk were analyzed from the herd at the North Carolina Experiment Station. The average amount of nicotinic acid was found to be 2.57 mg. per liter and the riboflavin content to be 1.82 mg. per liter. Colostrum was found to contain eight times as much of these two vitamins as did the later milk.

Light and Water in Growth and Competition of Piedmont Forest Tree Species. T. T. KOZLOWSKI.

The author reports on work indicating that physiological reactions of pine and oak seedlings under conditions of reduced light and soil moisture in competition are not similar. Yellow poplar and red maple reached maximum photosynthetic rates at lower light intensities than did loblolly pine. Absolute photosynthetic rates were 2 to 4 times as high for oak seedling as they were for pine. Potted pine seedlings grown in shade made less growth than did those grown in full light. Oak seedlings made excellent growth in both sun and shade. Subjecting pine and oak seedlings to drouth in sealed phytometers resulted in reduced dry weight increase. Oak seedlings had a greater capacity for absorption of water, when soil moisture was less than field capacity, than did pine seedlings. Pine in soil deficient in moisture absorbed more water at a high light intensity than in shade. As soil dried down photosynthetic rates of pine seedlings decreased more rapidly than those of white oak.

Effects of Fertilizer on Growth Rates of Ilex lucida. MARY G. CAUGHEY.

The effects of fertilizers on the growth of *Ilex lucida* (Ait.) T. and G., one of the shrubs typical of shrub-covered peat bogs of the southeastern coastal plain, were studied. Field tests were conducted in a shrub bog located in Beaufort County, North Carolina, between Chocowinity and Vanceboro. Soil factors, other than the high water table, which might be expected to affect the anatomy and physiology of bog shrubs are the low pH value and the low nutrient content of the peat soil. Analyses of the soil show it to be very deficient in nitrate and ammonium ions as well as in calcium ions and other exchangeable bases.

The purpose of this investigation was to study the effect of nitrate and calcium fertilizers on the growth rate of *Ilex lucida*. Seven plots 16 sq. yds. in area were used for the experiment. Two plots served as controls; four plots were treated with sodium nitrate at the rate of 200 pounds, 400 pounds, and 600 pounds per acre; one plot was treated with calcium sulphate at the rate of 2000 pounds of calcium carbonate per acre. On all the plots receiving the nitrate treatment shoot length was significantly greater than on the control plots. Sodium nitrate at the rate of 400 pounds per acre produced the greatest amount of growth; the average length of the main shoot and branches of shrubs receiving this treatment was five times and the average leaf area per sample was four times that of shrubs on the control plots. The shrubs, on the plot treated with calcium sulphate, were considerably stunted in growth; their shoot length and leaf area being only about one half that of shrubs on the control plots. It is concluded from these data that there is not sufficient available nitrogen in the peat soil for optimum growth of *Ilex lucida* under prevailing conditions. No explanation can be offered for the stunted growth of the shrubs on the plot treated with calcium sulphate.

North Carolina Gelidium Agars. CHRISTINE WILTON and HAROLD J. HUMM.

While making a study of the properties of agars from various red algae, extracts were prepared from two species of *Gelidium* which occur on the coast of North Carolina, namely, *G. crinale* (Turner) J. Agardh and *G. coerulescens* Keutzing.

The properties considered were viscosity, temperature of gelatin, and gel strength. Since species of the genus *Gelidium* are used almost exclusively in production of commercial agar, the North Carolina *Gelidium* agars were compared with Difco Bacto agar.

Gelidium crinale produced an agar with a slightly lower temperature of gelation than Difco Bacto but the gel strengths were about the same for both. *Gelidium coerulescens* produced an agar of unusually good quality, having a slightly lower temperature of gelation than Difco Bacto agar while the gel strength of a one per cent solution was 2.4 times that of Difco Bacto agar.

The Effect of Light Intensity on the Root Growth of Loblolly Pine. CHARLES W. BARNEY.

Three-month-old loblolly pine seedlings were grown under artificial light at 119, 290, 991, 5,330 foot candles and in complete darkness. The average daily root growth increased with increased light intensity above 290 foot candles. Light intensity of 119 foot candles was found to be too low for root growth. Lateral roots were formed and dormant roots commenced growth at 991 foot candles. A lag of about two days was observed between a change in light intensity and its effect on root growth. It is suggested that: (1) root growth of seedlings may take place at an adequate rate for the extension of new absorbing surface at light intensities as low as 290 foot candles provided soil moisture is not deficient; and (2) that winter dormancy of roots is apparently related to low light intensity as well as to low soil temperatures.

An Unusually Large Log from the Triassic. F. O. BOWMAN, JR.

An unusually large log, approximately seven feet long and one foot by one and one-half feet in diameter, was found in Triassic sandstone on the western border of the Durham Triassic Basin and was identified as a member of the Araucarineae tribe.

The Platiniferous Dunite of Yubdo, Ethiopia. T. G. MURDOCK.

(Published with permission of the former Chief, U. S. Technical Project in Ethiopia.)

The occurrence of platinum at Yubdo, in western Ethiopia, constitutes one of the best known mineral deposits in that country. Located within an area of pre-Cambrian crystalline rocks comprising the East African basement, the Birbir River and its tributaries have cut deeply and removed the volcanic covering, exposing a series of basic rocks enclosed in Algonkian schists. The basic rocks have a core of dunite which is surrounded by a belt of pyroxenites, and to the east the latter are succeeded by gabbros, in general constituting a geologic setting quite similar to that of the Ural platiniferous dunites. The dunite core is partially covered by "birbirite," composed essentially of quartz and iron oxide, and believed to be a product of the hydrothermal addition of silica, rather than the simple decomposition of dunite through the ordinary weathering processes.

The dunite is the host rock of the platinum although this is not visible nor directly exploitable in that rock. A concentration has been effected by the lateritic alteration of the platiniferous rock, and action of water on the altered rock. Thus there have been formed alluvial deposits, represented by the thick layer of laterite, and alluvial deposits composed of platiniferous gravels. The alluvials have in turn been transported by waters so that they are now found covering rocks other than the dunite. Their thickness ranges from 8 to 80 feet. Analysis of the platinum shows a similarity to that of the Urals. Average tenor for operations over a 10-year period was 0.155 gram per cubic meter.

Apart from a difference in original magmatic composition, the Yubdo dunite differs from those of western North Carolina in the mode of alteration, being characterized by laterization rather than serpentinization, and an almost complete absence, except for birbirite, of secondary silicates usually found associated.

Further Observations Concerning Carolina Bays. W. F. PROUTY.

Recent observations show that the "Carolina Bays" extend from the south Chesapeake Bay region to the northeast counties of Florida in the Atlantic Coastal Plain. In the extreme areas of both northeast and southwest occurrences, bays are less numerous and in small scattered groups. This more extensive distribution fits better the known distribution of meteoritic occurrences to the northwest.

A few "ghost bays" have recently been discovered in Georgia to the northwest of the Fall-line, as usually drawn. The "ghost bays" were apparently formed in Coastal Plain sand but have been, since their formation, largely eroded to the old crystalline floor. The area of their occurrence has many patches of only partially eroded Coastal Plain cover.

The axial orientation of the bays varies, more or less progressively, from about South 50° E. in the Norfolk region to about South 10° E. in the extreme southwest. This change in orientation is explained by the tendency of a vast number of bodies falling through an atmosphere to spread out as the result of pressure and heat developed in such fall.

Many peculiarly shaped bays result from infall of a number of meteorites in nearly the same locality. Some of the so-called multiple bays were apparently formed by a number of infalls following in rapid succession, from a tandem position, on the earth surface which is turning rapidly from west to east.

An attempt is also made in this paper to explain the varying orientations of the axes of the various units in this multiple bay.

Marls of Eastern North Carolina. E. W. BERRY.

Marls and limestones occur in the Pee Dee, Castle Hayne, Trent, Yorktown, Waccamaw, Chowan, and Pamlico formations in North Carolina. The marls of the Pee Dee (Cretaceous) seldom run over 32 per cent calcium carbonate. Those of the Castle Hayne (Eocene) range up to 88 per cent calcium carbonate. In the Miocene the Trent may go up to 90 per cent calcium carbonate while the Yorktown usually runs about half that. The Waccamaw (Pliocene) marls are often shell marls and although limited in extent sometimes run 95 per cent calcium carbonate. The Pleistocene marls in the Chowan and Pamlico formations usually do not run over 50 per cent calcium carbonate. There are thirty-five counties in eastern North Carolina which may have usable marls. Around New Bern and Wilmington are located the better deposits. The Simmonds pit on the Trent River and the quarry at Belgrade on the White Oak River are the only ones working at present (May 1947). Near Rocky Point on the North East Cape Fear and near Castle Hayne are fairly large deposits which might be worked. The present use is for road metal and steam cured lime brick, little or no marl being used on the land.

The Use of Compass Gradients in Geology. G. R. MACCARTHY.

In tracing magnetic formations by means of the "dial compass" it is customary to plot "dial reversal" or "magnetic" lines which are actually lines along which the declination reverses in sign.

It has been found that by plotting the gradients, or rate of change in the declination, rather than the declination itself, distinct advantages are obtained. The curves thus obtained are, compared to those showing declination, (a) less subject to errors in judgment, (b) less subject to the disturbing influence of adjacent formations, (c) do not require an accurate knowledge of the "average" local declination, and (d) more adapted to quantitative interpretation.

A Perched Triassic Trough in the West Wall of the Durham Triassic Basin. J. W. HARRINGTON.

Eastwood Lake northeast of Chapel Hill, Orange County, North Carolina, occupies a small valley whose floor is perched a few feet above the main floor of

the Durham Triassic Basin. Triassic sediment exposed as a discontinuous ring part way up the valley wall and a quarter of a mile west of main Triassic border outline in part the former extent of the once broader Triassic Basin. The western contact between conglomerate and granite has a gradational appearance and is sedimentary. The northern contacts indicate minor graben-like down faulting. Diabase dikes show some of the principal structural weaknesses which parallel the northern part of the Triassic scarp in that area. A platform of Carolina slates east of the Triassic remnant probably marks the floor of the pre-Triassic Valley in which the deposition took place.

Occurrence of Brackish Water in the Coastal Plain of North Carolina. M. J. MUNDORFF.

The occurrence of brackish water in some of the aquifers in the Coastal Plain is one of the most interesting ground-water problems in North Carolina. Most wells yielding such water are in the eastern half of the Coastal Plain, but a few are much farther inland and several of the wells are in crystalline rocks along the Fall Zone.

Most of the brackish water in the Coastal Plain is connate water—that is, water that was trapped in the sediments at the time of their deposition; or intrudent water—that is, water that entered them during a later geologic period.

Wells in the North Carolina Coastal Plain have encountered such water at depths ranging from 75 to about 600 feet. In some states fresh-water aquifers have been found below brackish-water aquifers but at no place in North Carolina is this known to occur. In this state, it is found that once connate or intrudent water is encountered in a well, the waters in the deeper aquifers are progressively more brackish.

Connate and intrudent waters are encountered at shallower depths adjacent to the major drainage channels than they are between these channels. The distribution of these shallower brackish areas shows a remarkable correspondence to the areal distribution of the lower Pleistocene terraces. This suggests that the strata in areas submerged during later Pleistocene time became saturated with sea water during the submergence.

A deep well at Enfield and one in Fremont, both in the Fall Zone, yield water from crystalline rocks. The water is very hard and is high in sulfate and chloride. Both wells are evidently yielding diluted sea water that was trapped in the rocks at some time when they were under the sea.

The well supplying the town of Fountain yielded water containing more than 300 parts per million of chloride when first drilled. The chloride content has declined steadily since the well was pumped. Evidently the dense granite mass which outcrops about 0.4 mile southeast of the well has impeded the circulation of ground water so that a pool of diluted brackish water remained trapped behind the granite hill after brackish water had been completely flushed out of adjacent strata. Fountain is nearly 20 miles east of the Fall Zone and the sediments there are about 400 feet thick; therefore, the granite mass must be a monadnock rising above the crystalline basement.

On the Inverse of Euler's Function. ALFRED BRAUER.

Let $\phi(t)$ be Euler's function. It is known only for a few values of n whether the equation $\phi(x) = n$ has solutions x. In this paper proof is given for Erdös' result that the equation $\phi(x) = n!$ has always solutions. This result is generalized as follows. Let k be any integer which is only divisible by primes less than or equal to n. Then the equation $\phi(x) = k \cdot n!$ has always solutions.

On Transon's Formula for Aberrancy. J. W. LASLEY, JR.

Abel Transon in 1841 developed the concept of aberrancy, or deviation—called by him the second "affection" of curvature. His discussion employs the terminology of infinitesimal polygons, consecutive points and relatively unknown properties of conics. He uses in his analytical argument the inclination of the tangent for independent variables rather than the arc-length of the curve.

If the latter is used, it is possible by means of a one-parameter family of equilateral hyperbolas to obtain his formula by direct calculus methods, and in a simpler form. It is the purpose of this paper to provide these details.

Is there a Dichotomy in Energy Mobilization? ELIZABETH DUFFY and OLIVER LACEY.

In psychology there appears to have grown up the curious assumption—not explicitly stated in these terms—that the individual functions at two distinct levels of energy mobilization, one the energy level of emotion, and the other the energy level of non-emotional conditions. The senior writer has repeatedly protested the dichotomizing of "emotion" and "non-emotion," and has suggested that the phenomena of emotion occur in a continuum, or rather in a number of continua.

Data will be presented to show that energy mobilization (as indicated by palmar skin conductance) is finely adjusted to the requirements of the situation, varying with the nature of the task, the degree of adaptation to the task, and the individual performing the task. The phenomena observed are more in harmony with a theory of continuous variations in energy mobilization than with a theory of a dichotomy between the energy mobilization of "emotion" and that of "non-emotion."

Influence of Study of Courses in Science on Development of Attitude Toward Evolution. K. L. BARKLEY.

College freshmen taking courses in introductory Biology, Chemistry, Mathematics, and both Biology and Chemistry were given the Thurstone scale on Attitude Toward Evolution at the beginning and at the end of these full year courses. A control group of commercial students who had a year of work in the college without any science courses was tested in the same manner.

Among the freshmen only those who took both Biology and Chemistry were significantly different from the others in attitude toward evolution when first tested, those taking both sciences being more favorable in attitude. Regular college freshmen, however, were significantly more favorable in attitude toward evolution than the commercial students.

During the year, all students of science changed to a more favorable attitude toward evolution, while the commercial students changed to a less favorable attitude. Only those students taking both Biology and Chemistry, however, made a statistically reliable change. The difference in degree of change was not statistically reliable between students studying the various sciences. The difference in degree of change between commercial students and students of science approached reliability in every case and was definitely reliable between commercial students and freshmen taking both Biology and Chemistry.

At the second testing, all groups of students who studied science were reliably more favorable in attitude toward evolution than the commercial students. Moreover, those students who had studied both Biology and Chemistry were reliably more favorable in attitude toward evolution than were those students who had studied only Biology, or Chemistry, or Mathematics.

A Statewide Wildlife Inventory. W. L. HAMNETT.

The North Carolina Division of Game and Inland Fisheries, through its Federal Aid Program, is conducting a statewide investigation of wildlife habitats and distribution. It is suggested that this project will assist in the development of practices which will result directly in the restoration and increase of our wildlife. Maps are being prepared to show the cover types in each of the counties of the state, and field men are placing the cover type data on these maps. Supplemental to the vegetational cover data is the relative abundance and distribution of game species found within the county. Information about the distribution and abundance of game species is obtained by personal observation and interviews with county residents. It is now proposed to have all the field data and map work of the state compiled into a final copy form which will become available to the people of North Carolina.

Principles of Wild Turkey Management. R. J. WHEELER, JR.

The application of a few, simple management techniques will usually result in transforming a heretofore unproductive territory into one that will carry and sustain a large density of wild turkeys provided the basic range requirement is at hand. This fundamental necessity is a sufficient amount of mature timber of the proper cover composition. Management is inexpensive to institute on such areas and if properly administered, is compatible with good land use. Range, on the other hand, which affords only a minimum of the requisite conditions, can be made to support a fair density but at a proportionately greater cost.

Optimum turkey range may be described briefly as a well-watered, forested area that is composed of a variety of timber types of which approximately 50 per cent is hardwood, one-half of which is mature oak, and that is broken by well-distributed forest clearings that support rank growth of native grasses, legumes, and plants that produce succulent fruits.

The well-managed forest clearing is the key to a successful wild turkey restoration program and is therefore the most gainful tool of the manager. The four principal native foods of the wild turkey, as based on annual consumption,

are: grasses, insects, mast and succulent fruits—hence the extreme importance of open land on turkey range.

Predator control is usually over-emphasized and often to the detriment of the game.

Minimization of human disturbance and the exclusion of live-stock and dogs are absolutely essential.

The Relation between Gill Surface and Activity in Marine Fishes. I. E. GRAY.

Among fishes there are species differences in number of gills, in number and length of gill filaments (primary lamellae), and in number of respiratory units (secondary lamellae). By determining the number and area of the respiratory units it is possible to obtain an estimate of the relative respiratory ability of different fishes.

There is marked contrast in relative respiratory surface between active, pelagic, migratory fishes (mackerel, menhaden, butterfish) and sluggish bottom fishes (flounder, toadfish, goosefish). The areas of respiratory surface of fishes of medium activity fall between these two extremes. The correlation between gill surface and activity holds true whether the gill area is compared with body weight or whether it is compared with body surface. A four-hundred-gram mackerel has a total of nearly three-fourths million respiratory units while a toadfish of the same weight has only fifty thousand. The number of respiratory units is also directly correlated with the amounts of sugar and hemoglobin in the blood. Seventeen species of marine fishes were studied.

The Correlation between Animal Size and Appendage Size in Habrobracon and its Use in Genetic Investigation. D. S. GROSCH.

In the parasitic wasp *H. juglandis* (Ashmead) there is a direct positive correlation between body size (as given by length of animal) and size of appendages (especially length). Evidence is furnished by analysis of materials from Grosch, 1945, by specific investigation of other established laboratory stocks, and by examination of experimentally produced dwarfs. These situations seem to be in accord with Huxley's formula of relative growth ($y = bx^k$).

Applications are as follows:

1. In view of this correlation it is important that animals of equivalent size be used in the study of expression and interaction of mutant genes. In many cases it becomes evident that genes with obvious major effect on the size and shape of one appendage have a measurable effect on other appendages. (Examples *sw* and *sb*)

2. In groups of normal offspring there is distribution of body size around the mean with appendage size varying accordingly. Body size depends on food supply. By demonstrating variation of antennal length in normal families to be thus dependent on an environmental factor, the correlation gives evidence of the genetic stability of secondary sex characters in *Habrobracon* (carried in the chromosome segment that functions as a new allele). Selection experiments over more than 50 generations failed to produce true breeding strains with

antennae of different length and segment number in established sex allele stocks (o e/f, +e/f, 25c, 25+).

3. The correlation can serve in classification of offspring from "two allele" crosses. Variations in antennal length accompanying normal slight variations in body size cause haploid and diploid male ranges of antennal segment number to overlap within and between stocks. However, if animals of equivalent size are compared, the number of antennal segments becomes technically the most feasible method of distinguishing haploid from diploid male, with diploid antennae made up of fewer but longer segments. (In practice it has been found useful to compare characteristics of a given animal with those of known haploids from virgin mothers of the same stock.)

Trombicula myotis, an American "akamushi". G. W. WHARTON.

Chiggers known commonly as "akamushi" in Japan have been incriminated as the vectors of tsutsugamushi disease in many parts of the Austro-Malayan and Oriental regions. Recently *Trombicula myotis* Ewing 1929 has been found to be a typical "akamushi."

Nesting Birds of Northwest Georgia. H. C. JONES.

Records of the birds of the Rome, Georgia, area cover the period from 1928 to 1931, and from 1934 to 1947.

The area studied most intensively lies in the southern end of the Appalachian Valley, at the junction of the Etowah and Oostanaula Rivers, headwaters of the Coosa and Alabama Rivers. The mixed hardwoods and pine "flatwoods" of the valley contain few bodies of water suited to aquatic or marsh species; most of the artificial ponds in the region are unprotected. "Victory Lake," on the Berry School campus, offers the best local waterbird habitat. Nesting sites for land birds, however, are numerous and varied. These range from the rocky slopes of the limestone ridges, forested with mixed hardwoods and pine, to the moist bottomlands which are forested with hardwoods such as elm, ash, maple, hickory and oak. Many birds find nesting sites in the numerous field margins, hedges, and the richly landscaped grounds of schools in the area.

A total of 184 species of birds have been recorded for this part of northwest Georgia. Of these, fifty-seven are known to nest, and thirty more probably nest, although we can find no record of their nesting.

Ontogenetic Variation in Certain Taxonomic Characters in the Dusky Salamander, Desmognathus f. fuscus. J. R. BAILEY.

Variation in the number of vomerine teeth and in certain body proportions of *Desmognathus fuscus* are discussed in relation to sex and size.

REINARD HARKEMA, Secretary.

CONSTITUTION OF THE NORTH CAROLINA ACADEMY OF SCIENCE

(With List of Officers and Members Appended)

ARTICLE I

NAME AND OBJECT

Section 1. The name of this organization shall be the "North Carolina Academy of Science."

Section 2. The objects of the Academy shall be to promote study and scientific research and to furnish, so far as practicable, a means of publication of such articles as may be deemed worthy.

ARTICLE II

MEMBERSHIP AND DUES

Section 1. (a) Members. Any person actively interested in science or the promotion of science, may, upon the payment of the annual dues of $2 and upon nomination by two members, be elected a member of the Academy by a majority vote of the Executive Committee, and shall be entitled to all the privileges of the Academy.

(b) Junior Members. Any person of high school grade who has shown unusual aptitude and accomplishment in some field of science may be elected to Junior Membership by a majority vote of the Executive Committee. Such membership is for the current year and there are no dues.

(c) Life Members. Members may be elected to life membership by the Academy upon nomination by the Executive Committee. Such election relieves the member from further payment of dues, but does not invalidate his right to present papers, vote, and hold office.

(d) Sustaining Members. Institutions, organizations, or industrial concerns may be elected as sustaining members by the Executive Committee upon the payment of a nominal fee. All funds collected from this source are to be placed at the disposition of the Research Grants Committee.

(e) Patrons. Any person contributing a sum of $10 or more annually to the Academy may be elected by the Executive Committee as a Patron. All funds collected from this source shall be at the disposal of the Research Grants Committee.

(f) Affiliates. Active organizations having scientific objectives may upon application and the payment of annual dues ($2.00) be elected by the Executive Committee as affiliates. The affiliation may be renewed from year to year by action of the Executive Committee. Affiliated organizations shall be entitled to receive the Journal or some substitute and such notices as are issued to the members of the Academy. Each such affiliate shall be entitled to elect a delegate to the Academy who shall have the right to vote.

Section 2. The annual dues for members shall be $2.00, and any person in arrears at the date of the annual meeting forfeits all privileges of the Academy.

ARTICLE III

OFFICERS

Section 1. The officers of the Academy shall be a President, Vice-President, Secretary-Treasurer, and an Executive Committee of six including the President, Vice-President, and Secretary, of which three shall constitute a quorum. The President and Vice-President shall be elected for one year. The Secretary-Treasurer, and three members of the Executive Committee for three years, one of these three members to be elected each year. All officials shall be elected, by ballot, by majority vote.

Section 2. The duties of all officers shall be such as usually pertain to such positions. The term of office shall begin with the adjournment of the meeting at which the elections are held and shall expire with the adjournment of the next regular annual meeting.

ARTICLE IV

REPRESENTATIVES

Section 1. The Academy representative to the American Association for the Advancement of Science shall be elected for a two year period.

ARTICLE V

MEETINGS

Section 1. The time and place of all meetings shall be determined by the Executive Committee, but there shall be at least one meeting annually for the presentation and discussion of papers, and at least one business meeting annually.

Section 2. Two weeks notice shall be given of all meetings, and those present at such meetings shall constitute a quorum.

ARTICLE VI

PUBLICATIONS

Section 1. The official organ of the Academy shall be the Journal of the Elisha Mitchell Scientific Society, published at the University of North Carolina. The Editor of the Journal and the Secretary of the Academy shall compose the editorial board for the Academy, subject to the general control of the Executive Committee of the Academy.

ARTICLE VII

AMENDMENTS

Section 1. This constitution may be amended by a two-thirds vote of those present at any regular meeting: Provided, that such amendments be submitted

in writing to the Executive Committee at least two weeks before the meeting at which action is to be taken.

BY-LAWS

1. The Executive Committee shall fill all vacancies occurring between meetings of the Academy.

2. Upon the written request of three or more members, the Secretary shall call a meeting of the Executive Committee, to consider such matters as may be laid before it, said meeting to take place within ten days from the time the request is submitted.

3. All elections to membership shall apply to the current calendar year, and no annual fee shall be collected for the year of such election.

4. The application for membership shall be accompanied by the initiation fee ($2.00), the same to be returned to the applicant should he not be elected.

5. Yearly dues regularly become payable on January 1st of each year.

6. The Secretary-Treasurer, during his term of office, shall not be liable for annual dues, and his necessary expenses in attending the regular meetings shall be defrayed from the treasury of the Academy.

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Vice-President, T. G. Pearson
Secretary-Treasurer, F. Sherman, Jr.

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Vice-President, C. E. Brewer
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1904

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1905

President, F. L. Stevens
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1907

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Vice-President, J. L. Lake
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1908

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Secretary-Treasurer, E. W. Gudger

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President, Tait Butler
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Secretary-Treasurer, E. W. Gudger

1910

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Secretary-Treasurer, E. W. Gudger

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Secretary-Treasurer, R. W. Leiby; Acting, C. S. Brimley

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Secretary-Treasurer, Bert Cunningham

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1944–1946

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1948

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Rice, Oscar Knefler, Chemistry, U. N. C.
Ryland, Alice Garnett, Biology, Coker College, Hartsville, S. C.
Satterfield, George Howard, Biochemistry, N. C. State
Scofield, Herbert Temple, Botany, N. C. State
Schumacker, F. X., Forestry, Duke
Seeley, J. Frank, Chemistry, N. C. State
Shaftesbury, Archie Davis, Biology, W. C. U. N. C.
Shands, Wayland Arthur, Entomologist, Orono, Maine.
Sherwood, Francis Webber, Animal Industry, N. C. State
Shields, James Duncan, Biology, Durham High School
Shunk, I. V., Botany, N. C. State
Simpson, Roxie Collie, Mrs., U. S. Natl. Mus., Washington, D. C.
Smith, Benjamin Warfield, Agronomy, N. C. State
Smith, Budd E., Botany, Wake Forest
Smith, Clyde F., Zoology, N. C. State
Smith, Frank Houston, Animal Nutrition, N. C. State
Smith, Juanita Maxine, Chemistry, N. C. State
Smith, Susan Gower, Medicine, Duke Medical School
Smith, Thomas Earle, McNair Seed Co., Laurinburg, N. C.
Smith, Wiley F., Psychology, Appalachian State T. C.
Speas, William Eugene, Physics, Wake Forest
Sponer, Hertha D. E., Physics, Duke
Stanland, Marion D., Psychology, N. C. Dept. Welfare
Stephens, James Linley, Lumberton, N. C.
Stevens, Ross Oliver, Secretary, N. C. Wildlife Federation
Stewart, Mary Josephine, Botany, Radford College, Radford, Va.
Strong, Cornelia, Mathematics, W. C. U. N. C.
Stuckey, Jasper Leonidas, Geology, N. C. State
Stuhlman, Otto, Physics, U. N. C.
Stump, Alex Bell, Biology, Flora MacDonald
Stupka, Arthur, Gatlinburg, Tenn.
Thiel, Albert Frederick, Botany, W. C. U. N. C.
Thies, Oscar Julius, Jr., Chemistry, Davidson
Thomas, Joseph Miller, Mathematics, Duke
Totten, Henry Roland, Botany, U. N. C.
Van Horn, Earl C., Geology, T. V. A., Murphy, N. C.
Vosburgh, Warren Chase, Chemistry, Duke
Walldroff, Bessie, Zoology, Elon
Warren, John R., Botany, Duke
Weaver, Richard Lee, Program Director, N. C. Resource-Use Education Commission, Chapel Hill.
Wharton, George Willard, Jr., Zoology, Duke
Wells, Bertram Whittier, Botany, N. C. State
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White, Raymond Cyrus, Chemistry, N. C. State
White, William Thompson, Jr., Microscopy, Bausch & Lomb, Washington, D. C.
Whitford, Larry Alston, Botany, N. C. State
Williams, David V. P., Oak Ridge, Tenn.
Williams, Leon Franklin, Chemistry, N. C. State
Williams, Louis G., Botany, Duke
Williams, Ruby M., Science-Chemistry, Durham High School
*Wilson, Robert North, Chemistry, Duke
Wilson, William, Physics, N. C. State

Wilton, Ocra Christine, Biology, E. C. T. C.
Wing, Merle Wesley, Zoology, N. C. State
Wolf, Frederick A., Botany, Duke
Wolf, Frederick Taylor, Biology, Vanderbilt, Nashville, Tenn.
Wolff, William A., Path. and Toxicology, Bowman Gray School of Medicine
Yarbrough, John A., Biology, Meredith
Yarbrough, Mary Elizabeth, Chemistry, Meredith
Yoder, Monroe Crain, Zoology, Lenoir Rhyne
Young, Elizabeth Barber, Education and Psychology, Greensboro College
Ziegler, A. W., Botany, U. N. C.
Zoology Field Club, W. C. U. N. C.

PROCEEDINGS OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY

1946–1947

450TH MEETING, OCTOBER 8, 1946

J. W. HUDDLE: *Mississippian Seas of Arizona.*

451ST MEETING, NOVEMBER 12, 1946

HAROLD HOTELLING: *Mathematical Statistics and Scientific Research.*

Dr. W. C. George was elected an associate editor of the Journal of the Elisha Mitchell Scientific Society to serve for the unexpired term of Dr. E. C. Pliske who had resigned.

452ND MEETING, DECEMBER 10, 1946

T. F. HICKERSON: *Area of a Field by Novel Methods.*

C. DALE BEERS: *A Comparative Study of Micronucleate and Amicronucleate Races of the Ciliate Tillina magna.*

453RD MEETING, JANUARY 14, 1947

J. P. GILLIN: *Witchcraft in Guatemala.*

454TH MEETING, FEBRUARY 11, 1947

L. O. KATSOFF: *Problems in Modern Logic.*

On motion of the editorial board of the Journal of the Elisha Mitchell Scientific Society, it was unanimously voted to create the post of Executive Editor of the Journal, the title and the name of the incumbent to be carried on the masthead of the Journal and the incumbent to serve as a non-voting member of the editorial board. Mrs. Alma Holland Beers was elected to serve as Executive Editor on permanent tenure.

455TH MEETING, MARCH 11, 1947

D. G. FREY: *The Fisheries of the Philippines.*

456TH MEETING, APRIL 8, 1947

H. E. ROBBINS: *Independent Random Events.*

457TH MEETING, MAY 13, 1947

W. A. WHITE: *The Origin of the Blue Ridge Scarp.*

The following officers were elected:

President (one year)—J. H. Ferguson.

Vice-President (one year)—Arthur Roe.

Recording Secretary-Treasurer (two years)—L. L. Garner.

CLAIBORNE S. JONES, *Sec.-Treas.*

ON THE IRREDUCIBILITY OF CERTAIN POLYNOMIALS*

By John O. Reynolds

Department of Mathematics, University of North Carolina, Chapel Hill, North Carolina

INTRODUCTION

In this paper "integral polynomial" shall mean a polynomial with rational integers as coefficients.

Let $a_1, a_2, \cdots, a_t$ be different integers, p a prime, $k \geqq 0$. The irreducibility in the field of rational numbers, P, of polynomials of degree n of the form

$$f(x) \equiv \prod_{\nu=1}^{t} (x - a_\nu)h(x) + p^k \tag{0.01}$$

has been studied in a large number of papers.

I. Schur [1908, 1909] proposed the following results as problems: The polynomials (0.01) are irreducible for $k = 0$, when $h(x) \equiv -1$; and if $t > 4$, for $h(x) \equiv 1$. These problems were solved by W. Flügel [1909].

H. L. Dorwart and Oystein Ore [1933] obtained the sharper result that the polynomials (0.01) are irreducible when the degree of $h(x)$ is less than t and $n \geqq 8$ for $k = 0$.

G. Pólya [1919] proved that the polynomials (0.01) are irreducible or the product of factors of equal degree, when $t = n$, if $n \geqq 17$, for $k = 1$. This bound was lowered by Dorwart and Ore to $n > 10$. A. Brauer [1933] proved that Pólya's theorem is true for $n > 6$, and showed by a counter example that this is the best bound for this class of polynomials.

For arbitrary k the following results were obtained by A. Brauer and R. Brauer [1935]:

Let $k \geqq 1$ be a positive integer and $p > 2^{k+2}$ be a prime number. If the integral polynomial $f(x)$, of degree n, takes the value p^k at more than $\frac{n + k^2 + 3k}{2} + 3$ integral points of which at least two are congruent (mod p), then $f(x)$ is irreducible in P. For $k = 1$ they obtained the sharper result: If the integral polynomial $f(x)$ of degree n takes the value $p > 5$ at t integral points of which at least two are congruent (mod p), then $f(x)$ is irreducible in P when $t > \text{Max}\left(\frac{n}{2}, 4\right)$. Moreover they proved that the polynomials (0.01) are irreducible in P or are the product of factors of equal degree when $a_1, a_2, \cdots, a_t$ are incongruent (mod p) for $n \geqq 13$ if $t > \frac{4}{5}n$. The cases $n \leqq 12$ were left undecided.

* This paper was presented to the faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the department of mathematics. The author wishes to express his appreciation for the guidance of Dr. Alfred T. Brauer in the preparation of this paper.

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In this paper we prove that the Brauers' theorem remains correct for $n = 4$, 5, 8, 10, and 12.

For $n = 7$ and $n = 11$, we cannot decide if the theorem is correct or incorrect. We do prove that the theorem holds if $t = n$.

That the Brauers' theorem is not true for $n = 3$ and $n = 6$, we shall show by giving counter examples.

For $t = n = 2m$, $k = 1$, H. L. Dorwart [1935a] derives necessary and sufficient conditions that the polynomials (0.01) be reducible into factors of equal degree. He gives examples for particular primes for $n = 8$, 10, and 12.

In Section 9 we prove that our polynomials are reducible into factors of equal degree only when these two factors take the one value 1 or -1 and the one value p^s or $-p^s$ for $t = n = 2m$ and $m \geqq 3$.

We derive necessary and sufficient conditions that the polynomials be reducible, which limit the values of the argument and put a condition on the prime power values taken as well.

It is trivial, that by multiplying Dorwart's polynomials by powers of p, that reducible polynomials are obtained which take the value p^k at n integral points. We show that non-trivial examples exist. We give such examples for $n = 2$ and $n = 4$ with arbitrary k and p, and for $n = 6$, 8 and 12 for $k = 2$ with particular primes p.

G. Pólya proved: If for n integral values of x, the integral polynomial $f(x)$ of degree n has values which are different from zero and, without regard to sign, less than $\dfrac{(n - [n/2])!}{2^{n-[n/2]}}$, then $f(x)$ is irreducible in P.

This result was improved for positive definite polynomials by H. Ille [1926], and for arbitrary polynomials by Tatuzawa [1939]. A. Brauer and G. Ehrlich [1946] obtained still a sharper result.

In this paper we improve Brauer's and Ehrlich's result for the case when the considered values of the polynomials are positive, with the following theorem: Let $f(x)$ be an integral polynomial of degree n. If for n integers x_1, x_2, $\cdots$, x_n

$$0 < \min_{x=1,\cdots,n} f(x) < \frac{(n-1)!}{2^{n-2}\{[(n-2)/2]!\}},$$

then $f(x)$ is irreducible in P.

1. *Some Lemmas*

(*1.1*) *Lemma*: If an integral polynomial $h(x)$ takes the values 1 and -1 at three integral points a_1, a_2, a_3, then the interval $\{a_1, a_2, a_3\}$ is of length less than 4.

Proof: We may assume without loss of generality that $h(x)$ takes the value plus 1 twice and the value -1 once, and also that $h(a_3) = -1$, $a_3 = 0$. Hence we can write

$$h(x) \equiv (x - a_1)(x - a_2)Q(x) + 1$$

Now $h(a_i) - h(0) = 2$ is divisible by $(a_i - 0)$, hence a_1 and a_2 are each divisors of 2. Therefore the length of the interval cannot be more than 4. To get an

interval of length 4, it is necessary that $a_1 = -2$, and $a_2 = 2$. If $Q(0) = 0$, then $h(0) = 1$ contrary to assumption. If $|Q(0)| \geqq 1$, then $|h(0)| = |-4Q(0) + 1| \geqq 3$, which contradicts $h(0) = -1$.

(1.2) Lemma: An integral polynomial may not take both values ± 1 at more than 4 integral points, and if 4 values ± 1 are taken the points are consecutive integers.

Proof: There are only four integers in an interval of length 3.

2. The Main Theorem

(1.3) Theorem: Let $f(x)$ be an integral polynomial of degree n that takes the value p^k, p a prime, at $t > \frac{1}{2}n$ integral points, a_ν, which are incongruent (mod p). Then $f(x)$ is either irreducible in P or the product of two irreducible factors of equal degree for $n = 4, 5, 8, 9, 10$, and 12.

Proof: Suppose that $f(x)$ is the product of two integral factors

$$f(x) = g(x)G(x) \tag{1.01}$$

where the degrees of $g(x)$ and $G(x)$ are m and M respectively, $(m \leqq M)$, then $n = m + M$ and

$$m \leqq \frac{n}{2} \tag{1.02}$$

Since $f(a_\nu) = p^k$, the possible values for $g(a_\nu)$ are ± 1, $\pm p^\lambda$, $0 < \lambda < k$, and $\pm p^k$.

Let α be the number of a_ν's for which $g(a_\nu) = \pm 1$, β the number of a_ν's for which $g(a_\nu) = \pm p^\lambda$, $0 < \lambda < k$, and γ the number of a_ν's for which $g(a_\nu) =: \pm p^k$. Thus

$$t = \alpha + \beta + \gamma > \tfrac{1}{2}n \tag{1.03}$$

For $\beta + \gamma$ of the arguments, $g(a_\nu) \equiv 0 \pmod{p}$. But, $g(x) \equiv 0 \pmod{p}$ has at most m incongruent solutions and

$$\beta + \gamma \leqq m \tag{1.04}$$

Similarly, $G(a_\nu) = p^k/g(a_\nu) \equiv 0 \pmod{p}$ for $\alpha + \beta$ a_ν's, thus

$$\alpha + \beta \leqq M \tag{1.05}$$

Adding (1.04) and (1.05) and applying (1.03) we get

$$\beta < \tfrac{1}{2}n \tag{1.06}$$

There are three cases to consider.

3. Proof of Case I

Let $g(x)$ take the same value c ($c = 1$ or $c = -1$) at α points, and also the same value d ($d = p^k$ or $d = -p^k$) at γ points. Form the polynomial

$$H(x) = G(x) + (p^k/cd)g(x) - p^k/c - p^k/d \tag{1.07}$$

If $H(x)$ is identically zero, then the factors $g(x)$ and $G(x)$ are of equal degree, $\frac{n}{2}$. If $H(x)$ does not vanish identically, then its degree is at most M. But, since $H(x) = 0$ for the $\alpha + \gamma$ a's.

$$\alpha + \gamma \leqq M \tag{1.08}$$

Since $g(x)$ can take the value c not more times than its degree

$$\alpha \leqq m \tag{1.09}$$

Using (1.04), (1.05), (1.08), and (1.09) we get

$$\begin{aligned} 5t = 5(\alpha + \beta + \gamma) &= \alpha + 2(\alpha + \beta) + 2(\alpha + \gamma) \\ &\quad + 3(\beta + \gamma) \leqq m + 2M + 2M + 3m = 4n, \end{aligned} \tag{1.10}$$

which contradicts $t > \frac{4}{5}n$, and case I is impossible.

Since case I admits factors of equal degree we assume for the remainder of the proof that $m < \frac{n}{2}$.

4. *Proof of Case II*

The case where $g(x)$ takes both the values 1 and -1. We shall discuss this case for each value of n separately, and shall denote n-II as we make the proofs for each n.

4-II. The only possibility is $m = 1$ and $M = 3$. From (1.03), (1.06), (1.04), and (1.05) we have $t \geqq 4, \beta = 0, \gamma \leqq 1, \alpha \leqq 3$. To have $t = 4$, it is necessary that $\alpha = 3$, but a linear factor cannot take the values ± 1 at three points, contradicting $t \geqq 4$.

5-II. $m = 1$ is impossible from 4-II. We have $t \geqq 5, \beta = 0$. $\gamma \leqq 2, \alpha \leqq 3$, where we need only to consider equality.

Due to the symmetry of values taken by a polynomial of degree two, and for $\alpha = 3$, we must have the minimum (or maximum) value of $g(x)$ to be -1 (or 1). By lemma (1.1) and the symmetry of $g(x)$, the values 1, -1, and 1 are taken at consecutive integral points. On the other hand $G(x)$ of degree 3 must take the values p^k, $-p^k$, and p^k at these same points and thus takes the values 1 and -1 twice each at non-integral points. Therefore $G(x)$ can take neither 1 nor -1 at more than one integral point, hence $\gamma \leqq 1$. Whence $t \leqq 3 + 1 = 4$, contradicting $t \geqq 5$.

8-II. By (1.03), (1.2), (1.04) and (1.06) we have $t \geqq 7, \alpha \leqq 4, \beta + \gamma \leqq m \leqq 3, \beta \leqq 1$. If $m \leqq 2$, we have a contradiction of $t \geqq 7$. Similarly for $m = 3, \alpha = 3$. For $m = 3, \alpha = 4$, by lemma (1.2) these four points are consecutive integers. We assert that $g(x)$ cannot take 1 twice and -1 twice. For suppose $x = -2, -1, 0$, and 1 and that $g(-2) = 1$, then we must have $g(-1) = g(0) = -1$ and $g(1) = 1$. Which gives $g(x) = (x + 2)(x - 1)(ax + b) + 1$. From $g(0) = -1$ we get $b = 1$, and from $g(-1) = -1$ we get $a = 0$ which is impossible. Hence $g(x)$ must take either 1 or -1 at three points. Assume that $g(x) = 1$ for $x = -2, -1, 1$ and $g(0) = -1$. This gives

$$g(x) = (x + 2)(x + 1)(x - 1) + 1.$$

$g(x)$ is increasing for all x outside the interval $\{-2, 1\}$; negative for $x < -2$, positive for $x > 1$, thus can take p^k and $-p^k$ only once each at integral points. At these two points $G(x)$ takes the values 1 and -1, and since the distance between these points is greater than 2 we have a contradiction. Therefore $\gamma \leqq 1$ and $t \leqq 4 + 1 + 1 = 6$ which contradicts $t \geqq 7$. A similar contradiction is obtained when we assume $g(x)$ takes the four values 1 and -1 in any possible order.

9-II. For this value of n we have $t \geqq 8, \alpha \leqq 4, \beta + \gamma \leqq m \leqq 4, \beta \leqq 1$. The only possibility here is $\alpha = 4, m = 4$. There are two cases.

(i) $g(x)$ takes 1 at 3 points and -1 at one integral point. By lemma (1.2) these points are four consecutive integers. Taking one of the four possible combinations of these values we may write $g(x) = (x + 2)(x + 1)(x - 1) + 1$. Outside the interval $\{-2, 1\}$, $g(x)$ is monotonic increasing hence can take p^k at not more than two integral points. The same is true for the other three combinations.

(ii) $g(x)$ takes the values 1 and -1 twice each, and can be written

$$(x + 2)(x - 1)(ax^2 + bx + c) + 1.$$

For $g(0) = g(-1) = -1$ we get $a = b$ and $c = 1$. For integral x outside the interval $\{-2, 1\}$, $g(x)$ is monotonic increasing for positive a and can take p^k not more than twice. (For negative a the value $-p^k$ can be taken only twice.)

In both cases $\gamma \leqq 2, t \leqq 4 + 2 + 1 = 7$, which contradicts $t \geqq 8$.

10-II. We have $\alpha \leqq 4, \beta + \gamma \leqq m \leqq 4$, thus $t \leqq 8$ which contradicts $t \geqq 9$.

12-II. For this value of n we have $\alpha \leqq 4, \beta + \gamma \leqq m \leqq 5, t \leqq 9$ which contradicts $t \geqq 10$.

5. *Lemmas for Case III*

In case III $g(x)$ takes both the values p^k and $-p^k$ but only the one value 1 or the one value -1 at integral points. Where $g(a_\gamma) = \pm p^k, G(a_\gamma) = \pm 1$, lemma (1.2) gives

$$\gamma \leqq 4 \tag{1.11}$$

*(1.4) Lemma:** If $g(x)$ of degree m satisfying (1.01) takes both the values p^k and $-p^k$ at $\gamma = 4$ integral points, then $\alpha \leqq m - 2$.

Proof: By lemma (1.2) for $\gamma = 4$ the points are four consecutive integers, thus $g(x)$ changes sign twice in the interval and takes the values ± 1 twice each at non-integral points. Since $g(x)$ may take a value at not more points than its degree we have the lemma, and

$$\gamma = 4, \alpha \leqq m - 2 \tag{1.12}$$

(1.5) Lemma: If $g(x)$ of degree m satisfying (1.01) takes both the values p^k and $-p^k$ at $\gamma = 3$ integral points, then $\alpha \leqq m - 1$.

Proof: Where $g(a_\gamma) = \pm p^k, G(a_\gamma) = \pm 1$, and by lemma (1.1) these points are contained in an interval of length 3. Since the difference in the points which

* For a different proof of this relation see Brauer and Brauer [1935].

give the values 1 and -1 is 2 or 1, two of the three points must be consecutive integers. Hence $g(x)$ changes sign at least once in the interval and takes the values 1 and -1 at non-integral points. $g(x)$ can then take neither 1 nor -1 at more than $m-1$ integral points, and

$$\gamma = 3, \alpha \leqq m - 1 \tag{1.13}$$

6. Proof of Case III

4-III. By (1.04) and (1.06) $\gamma \leqq 1$, hence case III does not apply to $n = 4$.

5-III. By (1.04) and (1.06) $\gamma \leqq 2$, and by (1.09) $\alpha \leqq 2$, thus we have a contradiction of $t \geqq 5$.

8-III. We have $t \geqq 7$, $\beta + \gamma \leqq m \leqq 3$, and $\alpha \leqq 3$. Thus $t \leqq 6$ which is a contradiction.

9-III. By (1.04) and (1.09) $\beta + \gamma \leqq m \leqq 4$, $\alpha \leqq 4$. For $m \leqq 3$, we have $t \leqq 6$. Since $\beta \leqq 1$, for $m = 4$ we have only two possibilities to get $t = 8$; for $\gamma = 4$ by (1.12) $\alpha \leqq 2$, thus $t \leqq 6$. For $\gamma = 3$, by (1.13) $\alpha \leqq 3$, hence $t \leqq 7$ which contradicts $t \geqq 8$.

10-III. Since $\beta + \gamma \leqq 4$ and $\alpha \leqq 4$, we get a contradiction of $t \geqq 9$.

12-III. By (1.06) $\beta \leqq 2$, from (1.11) $\gamma \leqq 4$, by (1.12) $\alpha \leqq 3$ for $\gamma = 4$. For $\gamma = 3$, (1.13) gives $\alpha \leqq 4$. Lastly for $\gamma \leqq 2$, by (1.09) $\alpha \leqq 5$, so in each case we obtain $t \leqq 9$ which contradicts $t \geqq 10$.

7. Polynomials of Degree 7 and 11

(1.6) Theorem: Let $f(x)$ be an integral polynomial of degree $n = 7$ or $n = 11$, that takes the value p^k at $t = n$ integral points which are incongruent (mod p), then $f(x)$ is irreducible in P.

Proof: Using (1.01), equation (1.03) now becomes

$$t = \alpha + \beta + \gamma = n \tag{1.14}$$

And (1.06) becomes

$$\beta = 0 \tag{1.15}$$

Case I of theorem (1.3) holds for theorem (1.6).

7-II. From (1.14), (1.04), (1.15), (1.02) and (1.2) we have $t = 7$, $\gamma \leqq m \leqq 3$, $\alpha \leqq 4$. For $m \leqq 2$ we obtain $t \leqq 6$, and for $\alpha = 3$, we get $t \leqq 6$ which contradicts $t = 7$. The only possibility for reducibility is $\alpha = 4$, $m = 3$. In section 4 8-II it was shown for $\alpha = 4$, $m = 3$ that $\gamma \leqq 1$ which gives $t \leqq 5$ and a contradiction.

11-II. For this value of n we have $\alpha \leqq 4$, $\gamma \leqq m \leqq 5$, thus $t \leqq 9$ which contradicts $t = 11$.

7-III. From (1.11) we have $\gamma \leqq 4$. For $\gamma = 4$ (1.12) gives $\alpha \leqq 1$; for $\gamma = 3$ (1.13) gives $\alpha \leqq 2$; for $\gamma \leqq 2$ (1.09) gives $\alpha \leqq 3$, in each case $t \leqq 5$ which contradicts $t = 7$.

11-III. From (1.11) and (1.09) we have $\gamma \leqq 4$ and $\alpha \leqq m \leqq 5$. Thus $t \leqq 9$ which contradicts $t = 11$.

8. *Reducible Polynomials of Degree 3 and 6*

That theorem (1.3) is not true for $n = 3$ and $n = 6$, is shown by the following examples:

$n = 3$: $\quad x(x+2)(x+p^k+1)+p^k = \{x+1\}\{x(x+p^k+2)+p^k)\}$

The construction of the example for $n = 6$, $k = 2$ is of sufficient interest to include here.

From the proof of 4-II in theorem (1.3) it is seen that $f(x)$ of degree 6 must break down into a quadratic and quartic. Under cases I and III of theorem (1.3) factorization is impossible, hence the construction under case II.

Since $\beta + \gamma \leqq 2$, and $\alpha + \beta \leqq 4$, we have two possibilities to get $t \geqq 5$: (i) $\alpha = 4$, (ii) $\alpha = 3$.

(i) Due to the symmetry of $g(x)$ of degree 2, and by lemma (1.2) for $x = -2, -1, 0, 1$, $g(x) = x^2 + x - 1$. We must have $\gamma \geqq 1$ and it is necessary for $x^2 + x - (p^2+1) = 0$ to have integral roots. This is impossible, since the discriminant $(2p)^2 + 5$ is not a square for $p \neq 1$.

(ii) $\alpha = 3$. By lemma (1.1) and the symmetry of $g(x)$, these three points are consecutive integers. Therefore $g(x)$ has a minimum value of -1 (or a maximum of 1). For $x = 1, 0, -1$, $g(x) = 2x^2 - 1$. If $g(a) = p^2$, then $g(-a) = p^2$, and $\gamma = 2, \beta = 0$. This gives $p^2 - 2x^2 = -1$, thus it is necessary that p be a solution of this non-Pellian equation. By hypothesis, the points are incongruent (mod p), thus $p \geqq 5$. The x solutions of this equation are $\pm\left(\frac{p^2+1}{2}\right)^{\frac{1}{2}}$. Consider now $G(x)$: $G(\pm 1) = p^2$ and $G(x) = (x^2-1)(ax^2+bx+c)+p^2$. From $G(0) = -p^2$, we get $c = 2p^2$. From $G\left(\pm\left(\frac{p^2+1}{2}\right)^{\frac{1}{2}}\right)$, we get $a = -4, b = 0$. Thus

$$f(x) = \{2(x^2-1)+1\}\{2(x^2-1)(p^2-2x^2)+p^2\}$$

$$= -8x^2(x^2-1)\left(x^2 - \frac{p^2+1}{2}\right) + p^2.$$

A particular example is obtained for $p = 7$ where the five points are $0, \pm 1, \pm 5$.

9. *Reducible Polynomials Taking the Value p^k at n Integral Points*

It follows from theorem (1.3) that the polynomials we are studying are reducible only as the product of factors of equal degree. We wish to study these reducible polynomials in this section.

(*1.7*) *Theorem:** Let $f(x)$ be an integral polynomial of degree $n = 2m$ that takes the value p^k at $t = 2m$ integral points, a_ν, which are incongruent (mod p). If $f(x) = g(x)G(x)$, where the degree of each factor is m, then $g(a_\nu) = p^k/G(a_\nu) = c$, $(c = 1 \text{ or } -1)$, for $\nu = 1, 2, \ldots, m$, and $g(a_\nu) = p^k/G(a_\nu) = d$, $(d = p^k \text{ or } -p^k)$, for $\nu = m+1, m+2, \ldots, 2m$, for all $m \geqq 3$.

* See Brauer and Brauer [1935] for proof for $m > 6$.

Proof: It is only necessary to show $f(x)$ irreducible under cases II and III of theorem (1.3).

Using our same notation, (1.15) and (1.14) give

$$\beta = 0, t = \alpha + \gamma = 2m \tag{1.16}$$

Since the factors are each of degree m, (1.2) and (1.09) give

$$\alpha \leqq \max\ (4, m) \tag{1.17}$$

$$\gamma \leqq \max\ (4, m) \tag{1.18}$$

Therefore, when $g(x)$ takes either both values ± 1, or both values $\pm p^k$, $t < 2m$ when $m > 4$. Thus we need only to prove our theorem for $m = 3, 4$.

For $m = 4$, it is necessary for $\alpha = \gamma = 4$, but in 9-II it was shown that for $\alpha = 4, m = 4$ that $\gamma \leqq 2$. In 9-III it was shown for $\gamma = 4, m = 4$ that $\alpha \leqq 2$, both of which contradict $t = 2m$.

For $m = 3$, it is only necessary to show $\alpha = 4$, and $\alpha = 3$ are impossible due to the symmetry of the degrees of the factors.

In 8-II it was shown for $\alpha = 4, m = 3$ that $\gamma \leqq 1$.

For $m = 3, \alpha = 3$, there is no loss in generality in assuming that $g(x)$ takes $+1$ twice. By lemma (1.1) these points are contained in an interval, I, of length 3. Setting $g(0) = -1$, the four possible polynomials $g(x)$ are:

$$(x + 2)(x - 1)(ax + 1) + 1 \tag{1.19}$$

$$(x + 1)(x - 2)(ax + 1) + 1 \tag{1.20}$$

$$(x + 1)(x - 1)(ax + 2) + 1 \tag{1.21}$$

$$(x - 1)(x - 2)(ax - 1) + 1 \tag{1.22}$$

In order to get $\gamma = 3$, it is necessary that at least two values $\pm p^k$ be taken by $g(x)$ at integral points outside I. We assert that this is impossible. These four polynomials are monotonic increasing or decreasing for all points beginning with the first integer outside I. Furthermore, if $g(x)$ is increasing to the left of I, it is decreasing to the right of I, and conversely, depending upon the sign of the coefficient a. Thus the value p^k may be taken only once on one side of I, and the value $-p^k$ only once on the other side of I. But $G(x)$ will take the values 1 and -1 at these same points. The difference in these points is greater than 3 which contradicts lemma (1.1).

We wish now to consider the factorable polynomials

$$f(x) \equiv A \prod_{r=1}^{2m} (x - a_r) + p^k = g(x)G(x)^* \tag{1.23}$$

where the degrees of the factors are equal.

* For $k = 1$, see Dorwart [1935a].

For $m \geqq 3$, we may write the factors in the following forms:

$$g(x) = B\prod_{\nu=1}^{m}(x - a_\nu) + 1 \tag{1.24}$$

$$G(x) = C\prod_{\nu=1}^{m}(x - a_\nu) + p^k \tag{1.25}$$

or

$$g(x) = B\prod_{\lambda=m+1}^{2m}(x - a_\lambda) \pm p^k \tag{1.26}$$

$$G(x) = C\prod_{\lambda=m+1}^{2m}(x - a_\lambda) \pm 1 \tag{1.27}$$

where in (1.26) and (1.27) like signs go together. Thus

$$A = BC \tag{1.28}$$

By (1.24) to (1.27) we have

$$B\prod_{\nu=1}^{m}(a_\lambda - a_\nu) = \pm p^k - 1, \qquad \lambda = m+1, m+2, \cdots, 2m \tag{1.29}$$

$$C\prod_{\nu=1}^{m}(a_\lambda - a_\nu) = -p^k \pm 1, \qquad \lambda = m+1, m+2, \cdots, 2m \tag{1.30}$$

Hence

$$C = \pm B = \pm\sqrt{|A|} \tag{1.31}$$

Equations (1.29), (1.30), and (1.31) give the

(1.8) Theorem: Necessary and sufficient conditions that (1.23) hold for $m \geqq 3$, are

$$\sqrt{|A|}\prod_{\nu=1}^{m}(a_\lambda - a_\nu) = \pm(p^k \pm 1), \qquad \lambda = m+1, m+2, \cdots, 2m \tag{1.32}$$

and the factors may be written in either of the forms (1.24)–(1.27).

Since (1.24) is identical with (1.26), we have the first $m - 1$ elementary symmetric functions of a_ν, $\nu = 1, \cdots, m$, equal to the same functions of a_λ, $\lambda = m+1, \cdots, 2m$ and that

$$B\prod_{\nu=1}^{m}(-a_\nu) + 1 = B\prod_{\lambda=m+1}^{2m}(-a_\lambda) \pm p^k \tag{1.33}$$

If a_1 be taken equal to zero, the problem of constructing polynomials of type (1.23) is equivalent to finding solutions of

$$\sum_{\nu=2}^{m} a_\nu^\mu = \sum_{\lambda=m+1}^{2m} a_\lambda^\mu * \qquad \mu = 1, 2, \cdots, m-1 \tag{1.34}$$

* For solutions of this system see Dickson [1920, 1929].

and primes satisfying

$$\prod_{\lambda=m+1}^{2m}(-a_\lambda) = \frac{(1 \mp p^k)}{B} \tag{1.35}$$

We give here examples of (1.23) for $A = 1, m = 1, 2$; and for $A = -1, k = 2$, $m = 3, 4$, and 6 for particular primes.

(1.36) $$m = 1$$

$$x[x \pm (p^k + 1)] + p^k = \{x \pm 1\}\{x \pm p^k\}$$

and

$$x[x \pm (p^k - 1)] - p^k = \{x \mp 1\}\{x \pm p^k\}$$

(1.37) $$m = 2, p > 5$$

$$x\left(x - \frac{p^k+5}{2}\right)\left(x - \frac{p^k+1}{2}\right)(x-2) + p^k = \left\{x\left(x - \frac{p^k+5}{2}\right) + 1\right\}\left\{x\left(x - \frac{p^k+5}{2}\right) + p^k\right\}$$

and

$$x\left(x - \frac{p^k+3}{2}\right)\left(x - \frac{p^k-1}{2}\right)(x-2) - p^k = \left\{x\left(x - \frac{p^k+3}{2}\right) - 1\right\}\left\{x\left(x - \frac{p^k+3}{2}\right) + p^k\right\}$$

(1.38) [Dickson, 1920, p. 705 (1)] $m = 3, p = 13, k = 2$

$$x(x-11)(x-1)(x-8)(x+3)(x-7) - 13^2 = \{x(x-11)(x-1) - 1\}\{x(x-11)(x-1) + 13^2\}$$

(1.39) [Dickson, 1920, p. 709, Bisman] $m = 4, p = 19, k = 2$

$$x(x+7)(x-6)(x+1)(x-3)(x-5)(x+4)(x+6) - 19^2 = \{x(x+7)(x-6)(x+1) - 1\}\{x(x+7)(x-6)(x+1) + 19^2\}$$

(1.40) [Dickson, 1920, p. 708, Tarry] $m = 6, p = 449, k = 2$

$$x(x-13)(x+9)(x-17)(x+5)(x-8)(x-5) \cdot (x+7)(x-16)(x+8)(x-15)(x-3) - 449^2 = \{x(x-13)(x+9)(x-17)(x+5)(x-8) - 1\}\{x(x-13)(x+9)(x-17)(x+5)(x-8) + 449^2\}$$

10. Improvement of a Theorem of Brauer and Ehrlich

Brauer and Ehrlich [1946, theorem 3] proved the following theorem. Let $f(x)$ be a polynomial of degree n with integral rational coefficients. If for

n integral values $x_1, x_2, \cdots, x_n$ the absolute value of $f(x)$ for $x = 1, 2, \cdots, n$ is less than

$$G = \frac{(n-1)!}{2^{n-1}\{[(n-2)/2]!\}}$$

but different from 0, then $f(x)$ is irreducible in the field of rational numbers.

We want to improve this theorem for the case when $f(x) > 0$ for $x = 1, 2, \cdots, n$.

(1.9) Theorem: Let $f(x) = a_0x^k + a_1x^{k-1} + \cdots + a_k$ be a polynomial of degree k, let $x_0 < x_1 < \cdots < x_k$ be $k+1$ numbers such that $f(x_\nu) \geqq 0$, $\nu = 0, 1, \cdots, k$, and let d_α be the length of the smallest interval which contains $\alpha + 1$ of these numbers ($\alpha = 1, 2, \cdots, k$). Then

$$\operatorname*{Max}_{\nu=0,1,\cdots,k} f(x_\nu) \geqq 2^{-k+1} \mid a_0 \mid d_1 d_2 \cdots d_k$$

Proof: For $k = 1$, $\mid f(x_1) - f(x_0) \mid = \mid a_0 \mid (x_1 - x_0) = \mid a_0 \mid d_1$
Since $f(x_\nu) \geqq 0$, we have Max $\{f(x_0), f(x_1)\} \geqq \mid a_0 \mid d_1$
This proves the theorem for $k = 1$.

Let us now assume that the theorem is true for polynomials of degree less than k.

Divide $f(x)$ by the polynomials $\prod_{\nu=0}^{k-1}(x - x_\nu)$ and by $\prod_{\nu=1}^{k}(x - x_\nu)$ respectively, then

$$f(x) = a_0\prod_{\nu=0}^{k-1}(x - x_\nu) + g(x) = a_0\prod_{\nu=1}^{k}(x - x_\nu) + h(x) \tag{1.41}$$

where $g(x)$ and $h(x)$ are polynomials of degree less than k with highest coefficients

$$b_0 = a_1 + a_0(x_0 + x_1 + \cdots + x_{k-1})$$

$$c_0 = a_1 + a_0(x_1 + x_2 + \cdots + x_k)$$

respectively. Now $c_0 - b_0 = a_0(x_k - x_0) = a_0d_k$, and

$$\begin{aligned} \text{Max } \{\mid b_0 \mid, \mid c_0 \mid\} &\geqq 2^{-1}(\mid b_0 \mid + \mid c_0 \mid) \\ &\geqq 2^{-1} \mid b_0 - c_0 \mid = 2^{-1} \mid a_0 \mid d_k \end{aligned} \tag{1.42}$$

But from (1.41)

$$\operatorname*{Max}_{\nu=0,1,\cdots,k} f(x_\nu) \geqq \max \left\{ \operatorname*{Max}_{\nu=0,\cdots,k-1} g(x_\nu), \operatorname*{Max}_{\nu=1,\cdots,k} h(x_\nu) \right\} \tag{1.43}$$

Since $g(x)$ and $h(x)$ are of lower degree than k, our theorem may be applied. Furthermore, the lengths of the smallest intervals which contain ν of the points $x_0, \cdots, x_{k-1}$ or ν of the points $x_1, \cdots, x_k$ are both not smaller than $d_{\nu-1}$. Hence

$$\begin{aligned} \operatorname*{Max}_{\nu=0,\cdots,k-1} g(x_\nu) &\geqq 2^{-k+2} \mid b_0 \mid d_1 d_2 \cdots d_{k-1} \\ \operatorname*{Max}_{\nu=1,\cdots,k} h(x_\nu) &\geqq 2^{-k+2} \mid c_0 \mid d_1 d_2 \cdots d_{k-1} \end{aligned} \tag{1.44}$$

By (1.44) and (1.42) we have

$$(1.45)\quad \operatorname*{Max}_{\nu=0,1,\cdots,k} f(x_\nu) \geqq 2^{-k+2}\, d_1 d_2 \cdots d_{k-1} \{\max(|b_0|, |c_0|)\}$$

$$\geqq 2^{-k+1} |a_0| d_1 d_2 \cdots d_k$$

In particular, from theorem (1.9), we obtain at once

(2.0) Theorem: Let $f(x) = a_0 x^{2m} + a_1 x^{2m-1} + \cdots + a_{2m}$ be a positive definite polynomial of degree $2m$, let $x_0 < x_1 < \cdots < x_{2m}$ be $2m+1$ numbers, and let d_α be the length of the smallest interval containing $\alpha + 1$ of these numbers, $(\alpha = 1, 2, \cdots, 2m)$. Then

$$\operatorname*{Max}_{\nu=1,\cdots,2m} f(x_\nu) > 2^{-2m+1} a_0 d_1 d_2 \cdots d_{2m}$$

(2.1) Theorem: Let $f(x) = a_0 x^k + a_1 x^{k-1} + \cdots + a_k$ be a polynomial of degree k, let $x_1 < x_2 < \cdots < x_n$ be a set of more than k integers such that $f(x_\nu) \geqq 0$, $\nu = 1, 2, \cdots, n$. Then

$$\operatorname*{Max}_{\nu=1,2,\cdots,n} f(x_\nu) \geqq 2^{-k+1} |a_0| \prod_{\lambda=1}^{k} \left[\frac{\lambda n - 1}{k}\right]$$

Proof: If we choose those $k+1$ of the integers x_ν whose subscripts are 1 and the k numbers $1 + \left[\frac{\lambda n - 1}{k}\right] (\lambda = 1, 2, \cdots, k)$, and denote these x_ν in increasing order by $x_0', x_1', \cdots, x_k'$, then [see Brauer and Ehrlich, 1946, pp. 847–8 for proof]

$$(1.46)\qquad d_\lambda' \geqq \left[\frac{\lambda n - 1}{k}\right] \qquad (\lambda = 1, 2, \cdots, k)$$

Applying (1.46) to theorem (1.9) we have

$$\operatorname*{Max}_{\nu=1,2,\cdots,n} f(x_\nu) \geqq \max_{\lambda=1,2,\cdots,k} f(x_\lambda') \geqq 2^{-k+1} |a_0| \prod_{\lambda=1}^{k} \left[\frac{\lambda n - 1}{k}\right]$$

By the proof of Brauer's and Ehrlich's theorem 3 [1946, pp. 848–50], making use of our theorem (2.1) instead of their theorem 2 [p. 847], their theorem 3 becomes

(2.2) *Theorem:* Let $f(x)$ be an integral polynomial of degree n. If for n integers $x_1, x_2, \cdots, x_n$

$$0 < \operatorname*{Min}_{\nu=1,\cdots,n} f(x_\nu) < \frac{(n-1)!}{2^{n-2}\{[(n-2)/2]!\}}$$

then $f(x)$ is irreducible in P.

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THE EFFECT OF A WATER-SOLUBLE CARCINOGEN ON NEURALIZATION IN AMBLYSTOMA EXPLANTS

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During the course of gastrulation in amphibia the invaginating roof of the archenteron causes the overlying dorsal ectoderm to differentiate as the neural tube and thus form the main axis of the embryo. Since invagination chiefly occurs at the dorsal lip of the blastopore, this region is called the "organizer" or sometimes the "primary organizer," despite the fact that its location, and consequently its potentialities, have been previously determined by extrinsic factors acting on the egg. The process of stimulus and response by which one region effectively influences the histological differentiation of another region is called induction.

The demonstration by Holtfreter (1933) that tissues capable of evoking an inductive response retained that capacity after being killed by boiling or treatment by various chemical agents, opened the way for chemical analysis and identification of the substance or substances responsible for induction in the embryo. Two important facts emerged early in the attempts at chemical analysis of induction: (1) The morphogenetic effect of the killed tissue or the chemical substance being assayed never duplicated the axial organization achieved by the living organizer; and (2) the parts of the gastrula, such as the ventral ectoderm or adult tissues of the most widely diverse sorts, which never normally possess inductive capacity, acquired it after being killed. Needham (1942) has perhaps done most in elaborating a widely accepted explanation of the second fact. According to this view, there is widely distributed in tissues, an inactive complex of the organizer substance which breaks down upon boiling or treatment with organic solvents and releases the heretofore 'masked' inducing substance. This release theory would then explain why variously killed tissues of diverse origin have a similar inductive effect on competent tissue. But it also raises another disturbing factor in the assay of all dead tissues and chemical substances which are being tested for their inductive capacities. It would be impossible in any given case to be sure that the substance being tested has not injured the cells of the responding tissue and so liberated the active inducing substance present in it. If an induction occurs under these circumstances, it may as likely be the result of indirect induction, due to the noxious effect of the material being tested on the responding tissue with release of the true inducing substance from the injured cells, as it is to be the result of a direct induction brought about by a substance within the implant which acts directly on the cells of the responding tissue in such a way as to change the course of their normal differentiation. Unless this uncertainty as to possible interference of injury and indirect release of active inducing substance in the inducing process can be removed, the way to chemical analysis of the inducing substance appears to be effectively blocked.

Special interest in the behavior of *Amblystoma punctatum* ectoderm arises, not only because of the frequent use made of the eggs of this species in experimental work but also because of what appears to be its exceptional sensitivity to noxious agents. Barth (1941) discovered that large pieces of isolated gastrular ectoderm of *A. punctatum*, cultured in 0.38% salt solution, frequently differentiated into neural tissue in the absence of an organizer. This aberrant behavior, so different from that of other amphibian species, was confirmed by Holtfreter (1944, 1945). From the observation that the inner, uncoated side of the *Amblystoma* explants, after being exposed for about 8 hours to the standard saline medium, disintegrated into single cells which subsequently underwent cytolysis, he concluded that the inorganic culture medium itself which had previously been considered to be neutral, i.e., to have no specific effect upon differentiation, was just another neural "inductor" as far as *A. punctatum* ectoderm was concerned. The cytolysis of the cells of the inner coat of the ectodermal pieces, which was indicated by the shedding of dead cells from the explant was, Holtfreter thought, causally related to the neural induction which frequently resulted in the explant. "Under conditions of increased permeability and chemical decomposition, certain substances have become released which entered adjacent cells of the aggregate and initiated neuralization." Although apparently only in *Amblystoma* does the extreme sensitivity of the inner ectodermal coat to the standard saline medium result in cytolysis, release, and neuralization in the complete absence of foreign tissue, nevertheless, the same sequence of events may be suspected in other species where dead tissues or chemical substances have produced neuralization.

Shen (1939) suggested that a study of dosage offered a means of distinguishing between direct and indirect forms of induction. If the dose of a substance X which produces neuralization is very small there is less probability that it is acting by first cytolyzing the ectodermal cells and so causing the release of the active inducing substance of the ectoderm itself. If its effect were indirect, induction would be expected to increase with concentration, reaching a maximum beyond which the increasing toxicity would prevent induction because of extensive tissue damage.

Shen tested the hypothesis by implanting in carefully graded doses into the blastocoel of gastrulae of *Triton alpestris* the water-soluble carcinogenic hydrocarbon, 1,2,5,6-dibenzanthracene-α-β-endo-succinate. Waddington (1938) had previously tested the compound as an inductor and found it very active, giving 39% neural tube inductions. Shen found an optimal dose of the hydrocarbon to be 0.0125 mgm. per embryo. At this dilution the number of neural tubes induced was a little over 40% of the number of embryos in which the implant was retained; on either side of this dilution the percentage of neuralization fell off. Shen suggested that his results indicated, for the reasons given above, that the action of this hydrocarbon was direct and imitated the action of naturally occurring inducing substance and not indirect, by cytolysis and release of the masked inducing substance. Needham (1942) considered the most important point about Shen's investigation to be the low level of dose required to produce neural induction. He found by calculation that this optimal dosage lay in the same range as that of many biological stimulating substances such as hormones, vitamins,

estrogens and carcinogens and believed that this fact made it seem more likely that the action of the hydrocarbon was direct rather than indirect.

In a later paper, Shen (1942) reported the results of cultivating small pieces of isolated presumptive epidermis of early gastrulae of *Amblystoma mexicanum* for 10 days, each in 1 cc. standard saline medium containing 10 mgm./cc. of the water-soluble hydrocarbon used in the previous investigation. 16 of 25 control pieces cultured in saline alone survived and were found on histological examination to be wholly epidermal. 18 of 25 experimental pieces survived and 11 of these showed masses of neural tissue. No evidence of toxicity was seen in either series. "By avoiding the use of solid implant and thus eliminating any possible mechanical role of an inductor, it proves clearly that, as far as evocation is concerned, the chemical action alone of the evocator constitutes an adequate stimulation."

The investigation reported in the present paper was undertaken in the hope of demonstrating in *Amblystoma punctatum* ectoderm the optimal dose effect of the same chemical agent used by Shen. The work of Barth (1941) and of Holtfreter (1945) had made it necessary that all previous experiments with foreign inductors be reconsidered from the viewpoint of cytolysis, although Van Cleave (1947) had indicated that by the use of the "sandwich-type" of ectodermal explant in *A. punctatum* the presumptive cytolytic effect of the saline medium could be minimized.

MATERIAL AND METHODS

In these experiments the inductive effect of the water-soluble hydrocarbon was tested by the method of explanation. The implantation mass containing the material to be tested was placed between two pieces of ectoderm which quickly fused to form an unbroken outer coat of ectoderm. The ectoderm used came from gastrulae of *A. punctatum:* in one series of experiments the donor eggs corresponded to Harrison's stage 10 (slit blastopore) and in another, stage 12 (medium yolk-plug). After orienting the egg, a small area of ectoderm, 180° opposite the blastopore was removed and always carefully cleaned, if necessary, of adherent cells. In the earlier stage of gastrulation, this ectoderm was presumptive belly epidermis plus presumptive anterior medullary plate: in the advanced stage of gastrulation, the ectoderm was taken from the area of presumptive epidermis only. In all explants both top and bottom pieces of ectoderm came from the same egg. All operations were done with a sterile technique.

Previous experience with stage 10 ectoderm had shown (Van Cleave, 1947) that under the routine experimental conditions by which the inner ectodermal coat is only briefly exposed to saline solution, and in the absence of heterogeneous cells or materials, explants of presumptive epidermis and of presumptive anterior medullary plate differentiated as pure epidermis. In explants of ectoderm from eggs of a more advanced stage of gastrulation, neuralization had been encountered; in one series of 31 explants from stage 12, for example, 29 differentiated as epidermis, 1 showed neuroid tissue and 1 a small neural mass without structure.

The material tested as an inductor in these experiments was Na-1,2,5,6-

dibenzanthracene-9,10-endo-α-β-succinate (trans). Dibenzanthracene was obtained from the Eastman Kodak Company. The double Na salt of the succinic acid derivative was prepared for me by Messrs. Joyner and Bennett of the Department of Chemistry, University of North Carolina, according to the methods of Bachmann and Kloetzel (1938) and Warren (1939). The concentration of the stock solution was 4.7 mgm. per 1 cc. of distilled water, which represented the maximum solubility of our material. All successive dilutions were made from this stock solution. When dilution was tried with standard Holtfreter saline solution (containing NaCl, KCl, $CaCl_2$) a turbidity appeared which developed into an appreciable precipitate on standing overnight. A clear solution was obtained with an aqueous 0.35% NaCl solution. Turbidity occurred only in the presence of Ca, suggesting a salting-out effect. Consequently all subsequent dilutions of the stock solution of DBAS were made in 0.35% NaCl solution. The pH of all concentrations used was checked and found to be approximately 7.6.

The method of preparing the DBAS for implantation followed Shen's procedure. Crystalline egg albumin Merck was added, in the proportion of 100 mg. to 1 cc., to the DBAS dilution being used and after thorough mixing, was coagulated by brief immersion of the container in boiling water. By means of an ocular micrometer, a piece of coagulum approximating 0.25 cmm. in volume was cut from the mass as each explant was prepared. Since the concentration of the DBAS was known, a uniform dosage per implant for each dilution can be obtained. The implantation mass was made up fresh before each set of explants was prepared. After 10 days of isolation at room temperature, all explants were fixed, sectioned and examined microscopically.

The concentration of DBAS per dose may be briefly tabulated as follows: The stock solution of DBAS contained 4.7 mg. per cc. This was the strongest dose given and corresponds to Shen's strongest dose of 5.53 mg./cc. Six successive dilutions of the original stock solution were made at dilutions of 10. After adding egg albumin, 1 cmm. of the implantation mass contained approximately 5 gamma of the hydrocarbon in the case of the stock solution and since the average implant was 0.25 cmm., the strongest dose per explant was 1.25 gamma.

SERIES	CONCENTRATION	DOSE PER IMPLANT
	mg./cc.	mg.
DBAS #1	4.7	1.25
2	.47	.125
3	.047	.0125
4	.0047	.00125
5	.00047	.000125
6	.000047	.0000125
7	.0000047	.00000125

EXPERIMENTAL RESULTS

The effects of implants containing known, uniform amounts of DBAS on explants of competent ectoderm as shown by microscopic examination after cul-

tivation of the explants are recorded in Tables I and II. Table I represents the response of late gastrular ectoderm to a series of 5 concentrations of DBAS ranging in dosage from 1.25 mg. per implant to .000125 mg. Table II records the response of early (Stage 10) ectoderm to dosages of DBAS extending from the previous strongest dose, 1.25 mg. to two further dilutions, the weakest being .00000125 mg. per implant.

TABLE I

Neural induction in explants of late gastrular (stage 13) ectoderm of A. punctatum with implants of DBAS in egg albumin

SERIES	EXPLANTS EXAMINED	IMPLANTS RETAINED	RESPONSE:		
			Epidermis	Neuroid	N.T.
Egg Albumin only	10	9	8	1	
DBAS #1	11	11	11		
2	9	8	7	1	
3	9	9	8		1
4	9	7	4	3	
5	10	8	7	1	

TABLE II

Neural induction in explants of early (stage 10) gastrular ectoderm of A. punctatum with implants of DBAS in egg albumin

SERIES	EXPLANTS EXAMINED	IMPLANTS RETAINED	RESPONSE:		
			Epidermis	Neuroid	N.T.
Egg Albumin #1	15	14	7	7	
Egg Albumin #2	20	20	18	2	
DBAS #1	13	12	7	5	
2	16	16	14	2	
3	14	14	14	0	
4	9	7	7	0	
5	13	13	13	0	
6	10	10	9	0	1
7	14	13	10	0	3

Microscopic examination of such isolated ectodermal explants shows one of two alternative pictures: either the ectoderm has wholly differentiated as epidermis or some part of the otherwise epidermal mass has differentiated as histologically recognizable neural tissue. The latter alternative, however, presents a gradation of histological effects which have been considered early or "weak" degrees of neuralization. A probably advanced form of this transitional process between indifferent ectoderm and histologically recognizable nervous tissue is a localized differentiation characterized by the elongation and orderly lining-up of cells, perpendicular to free internal surface. Such tissue has been called "palisades" or "neuroid", although evidence that this is a developmental stage in the differ-

entiation of neural tissue is lacking. Histologically recognizable neural tissue appears in explants in quantity ranging from small spherical masses, irregularly disposed and usually sharply delimited from the surrounding epidermis to short neural-tube-like structures and large thick-walled brain masses with associated optic vesicles and nasal placodes. In the present investigation, the occurrence of neuroid tissue is recorded as well as indubitable neural tissue. No other tissue differentiation was observed.

Implants of egg albumin with the usual proportion of 0.35% NaCl were tested with ectoderm of both stages of gastrulation. With stage 12 ectoderm, 8 of the 9 explants which had not extruded their implant showed only epidermis present; one of the 9 showed neuroid tissue in contact with the implant. Two sets of explants of stage 10 ectoderm are available as albumin controls. In one set of 20 explants, made of ectoderm from eggs from a different geographical source, 2 showed neuroid tissue, the rest were epidermal. In another set of 15, made from eggs from the same source as those treated experimentally with DBAS, 7 of the 14 explants which retained their implants showed palisades. In no albumin control did neural tissue appear.

From the results recorded in Tables I and II, it is evident that DBAS in the various concentrations used did not induce the formation of neural tissue in the gastrular ectoderm which was tested. In the 43 explants of late gastrular ectoderm which might be expected to respond, only one example of neural tissue in the form of a small neural-tube-like structure was found. In explants of the earlier gastrular ectoderm, neural tissue is first encountered when the concentration of DBAS becomes almost homeopathic. Palisades appear at first to parallel the concentration but this is unlikely to be an effect of DBAS since albumin alone in the absence of DBAS, had produced neuroid tissue in more explants. To indicate the potential response of these stages of gastrular ectoderm in explants, it might be pointed out that Van Cleave (1947) reported 63% neuralization of stage 12 ectodermal explants containing an implant of a dead optic cup and 57% neuralization of stage 10 ectoderm with the same material.

Sections of all explants were carefully examined for possible evidence of carcinogenic activity. Nothing that could be interpreted as accelerated growth or as toxic effect on cells was seen. Pyknotic nuclei were occasionally seen but were no more numerous in explants treated with DBAS than in albumin controls. The few explants in which neural tissue was found showed less cellular injury than many explants which were entirely epidermal.

DISCUSSION

Under the experimental conditions described above, the water-soluble double Na succinate derivative of dibenzanthracene failed to induce neuralization in explants of *A. punctatum* gastrular ectoderm. This is contrary to the experience of both Waddington (1938) and Shen (1939) who used approximately comparable procedures. A major difference lies in the different amphibian species used, *Triton alpestris* vs. *Amblystoma punctatum*. Holtfreter's characterization of the unique susceptibility of the gastrular ectoderm of the latter species has been

mentioned above although in the present, as in former experiments, the inductive consequences of the suggested injury and release theory have failed to appear. Nevertheless, the specific difference is very real and to it may be due the difference in results.

Although there is no evidence in these experiments that DBAS has influenced competent ectoderm to differentiate as neural tissue, the absence of neural differentiation in what is known to be potentially very responsive tissue must be examined as a possible inhibitory effect of the carcinogen. The statement is frequently made that malignant cells are less differentiated than normal ones; an antagonism is assumed between growth and differentiation and carcinogens might be expected to act in part by inhibiting differentiation. It has been previously mentioned that no signs of accelerated growth were seen in the experimental explants but can the absence of neural differentiation be ascribed to an impeding effect of the DBAS?

Briggs and Briggs (1943) studied the effects of continuous exposure to DBAS on early development of eggs of *Rana pipiens*. The concentrations used were 0.02, 0.213, 2.13, 4.26 and 10.0 mgm. per 100 cc. Embryos exposed to the two strongest solutions disintegrated without completing neuralization, the degeneration following a uniform pattern of differential susceptibility. Embryos exposed to the 2.13 concentration developed satisfactorily but showed significant retardation of growth rate and of development of function, as indicated by capacity for muscular movement during post-neurula development. No evidence of stimulative effect of the carcinogen was seen in any concentration. Most pertinent to the present discussion was the lack of any effect on the morphological organization of the embryo. Experiments with various noncarcinogenic agents indicated to the authors that the effects observed were specific for DBAS and suggested that the carcinogenicity of DBAS might be responsible for the effects. Other workers have reported both retardative and stimulative effects of carcinogens on such materials as tissue cultures and various invertebrates but the extent to which such results can be compared with amphibia is limited.

It is unlikely that the specific retardative effect of DBAS on the whole egg of *Rana pipiens* which is suggested by Briggs and Briggs offers an explanation of the absence of neural differentiation in the explants of *A. punctatum* treated with DBAS. Implants of the egg albumin implantation mass alone were as ineffective as neural inductors as implants containing the strongest concentration of DBAS. Perhaps the situation here parallels that observed in other experiments with explants of gastrular ectoderm of *A. punctatum* which contained dead larval liver fragments (Van Cleave, 1947). This denatured material induced only 4.3% neural tissue (2 explants of 39) in stage 10 ectoderm that with implants of dead optic cup and dead hind-brain material responded with 57% and 80% neuralization respectively. In the case of these inductively ineffective materials, there seems no grounds for assuming a hypothetical inhibitory process. The alternatives might be the absence of noxious agents, either qualitatively or quantitatively, which prevents the release of a masked inductor or the absence of a substance or substances in the implant which directly induces neural differen-

tiation. The evidence from the explant method described here seems to me to favor the latter alternative.

SUMMARY

Gastrular ectoderm of *Amblystoma punctatum* in the form of sandwich-type explants was exposed to implants of egg albumin coagulum containing known amounts of Na-1, 2, 5, 6-dibenzanthracene-9, 10-endo-α-β-succinate (trans). The dosage per implant ranged from 1.25 mg. to 0.00000125 mg. After 10 days of isolation, the explants were killed, sectioned and examined microscopically.

The results were as follows:

1. There was no evidence that the water-soluble carcinogen induced neuralization in the presumptive epidermis.

2. Neither acceleration nor retardation of growth was observed in the explants subjected to any of the concentrations of DBAS which were used.

The relation of these results to the suggested theory of indirect induction is discussed.

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THE RELATION OF DENSITY OF POPULATION TO ENCYSTMENT IN DIDINIUM NASUTUM[1]

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Experimental evidence shows conclusively that encystment in the holotrichous ciliate *Didinium nasutum* can usually be induced by absence of food, although the response of didinia to lack of food is somewhat unpredictable, in that the animals may fail to encyst and may die of starvation (Mast, 1917). However, encystment is sometimes associated with an abundance of food, as Mast also observed. Indeed, Mast and Ibara (1923) concluded that didinia actually encyst more readily in cultures which are supplied with food than in cultures which lack it. They attributed their results to the greater accumulation of metabolic waste in such cultures. Since the didinia multiplied rapidly prior to encystment, it was assumed that the metabolic waste responsible for encystment was a product of the didinia themselves.

The writer (1927) subjected many groups of didinia, each consisting of 5 well-fed specimens of the same clone, to absence of food and on the basis of actual counts of individuals calculated the percentage of encystment. Each group was deprived of food in 0.2 cc. of spring water in the concavity of a depression slide. Since a well-fed didinium continues to divide in the absence of food, as Mast (1909) pointed out, each group produced 10 or more progeny. Of these, some usually encysted, whereas others remained active, eventually to die of starvation. In one typical series of experiments (loc. cit., p. 502, Table 1) 375 didinia of "Clone 1" produced 799 progeny, only 346 or 43.3 per cent of which encysted. Other clones gave similar results. If the unencysted specimens were fed and allowed to multiply, their progeny upon being subjected to a second starvation test repeated the history of the first test. Hence, the unencysted individuals were not fundamentally different physiologically from those that encysted. In various tests involving many hundreds of individuals the number that encysted usually varied from 40 to 55 per cent. The remarkable feature of these experiments was the fact that the didinia responded differently—some encysted, others remained active—even though they were members of a single clone and were subjected to the same cultural and experimental treatment.

It is evident from the foregoing that the precise relation of encystment to absence of food in *Didinium* has not been satisfactorily demonstrated, in spite of the efforts of a number of investigators. This circumstance has led me finally to investigate a further aspect of the problem, namely, the relation of density of population or crowding to encystment in the absence of food. This study was prompted by a reexamination of the results of Barker and Taylor (1931) on the

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relation of crowding, both with and without food, to encystment in *Colpoda cucullus*. They found that as the concentration of colpodas in a given amount of medium was increased the time required for encystment was decreased. For example, when the average number of colpodas per 0.5 cc. of medium (non-nutrient and containing no bacterial food) amounted to 17.7, nearly 8 days were required for 50 per cent encystment; when the number was increased to 159 only 2 days were required, and when the number reached 1800 or more less than 1 day was required (p. 632, Fig. 4 and Table 5). Cultures which contained food had a similar history, though a slightly longer time was necessary for encystment. Their evidence indicated (p. 633) that "food is evidently a factor of quite secondary importance" in inducing encystment and that "crowding and the alteration of the environment due to the accumulation of metabolic products" of the ciliates are the factors of primary importance. These conclusions suggest that perhaps the failure of many (half or more) of the didinia to encyst when deprived of food in groups of 5 (Beers, 1927) or of 10 (Beers, 1927a) was due to insufficient crowding, i.e., to the presence of a set of conditions which constituted a weak or only partially adequate stimulus to encystment. Therefore, in the present study larger numbers of didinia were deprived of food, many in 0.2 cc. volumes of various fluids as formerly, and some in smaller volumes, as a means of ascertaining the relation of density of population or crowding to encystment.

METHODS

Two clones of *Didinium*, each the product of an excysted specimen, were used in the study. The progenitors of these cysts were collected in 1945 in the vicinity of Chapel Hill, North Carolina. Pure lines of each were cultured in spring water in depression slides to which ample numbers of paramecia (*P. multimicronucleatum*, washed and concentrated in spring water) were added as food. Small stock cultures prepared with surplus animals from the lines furnished didinia for the encystment tests. All didinia used in these tests were full-grown, well-fed specimens of reasonably uniform size. By such a specimen is meant an individual which measures approximately 140 μ by 115 μ and which is about 4 hours removed from the preceding division. At 22°C. *Didinium* usually divides every 6 hours, other conditions being favorable. By isolating and culturing the daughters immediately after division, specimens precisely 4 hours old may be obtained, but the method is scarcely practicable when large numbers of didinia are needed. Even these specimens will vary in size and in the number of fissions (1 or 2) which they will undergo in the absence of food, depending on how recently a paramecium has been ingested. Hence, this method of obtaining didinia of uniform age and size was not employed. With experience well-fed individuals of uniform size can be picked out of a mass culture almost as accurately as they can be obtained by individual isolation and culture.

Although relatively large numbers of didinia (300 per 0.2 cc.) were needed for some of the experiments, the centrifuge was not employed as a means of concentrating them. Didinia sediment slowly, when compared for example with paramecia, and seem actually to oppose the centrifugal force, for they are strong swimmers. Force sufficient to throw them to the bottom of the tube usually

flattens them excessively. Within limits such specimens will usually recover and live normally, but their use in encystment studies seemed inadvisable. Therefore, when large numbers of didinia were needed for isolation in small volumes of fluid, they were concentrated by filtration. A piece of glass tubing having a length of 1 cm. and an inside diameter of 6 mm. was expanded slightly at one end. Over the other end was tied a piece of silk bolting cloth which had apertures measuring 40–45 μ square. Thus there resulted a small filtration dish with a bolting-cloth bottom and with an expanded top to facilitate the final removal of the didinia. In use the bottom of the dish rested on small glass rods suitably bent and arranged to stay in place in a Syracuse watch glass. The watch glass and filtration dish were filled—obviously in one operation—to a depth of about 4 mm. with fluid of the kind in which the didinia were to be deprived finally of food, for example, spring water. By means of a small pipette didinia were put into the filtration dish in numbers somewhat in excess of the anticipated experimental needs. By adding spring water dropwise to the filtration dish and withdrawing it from the watch glass any desired amount of fluid could be passed over the didinia as a means of washing them and getting them into pure spring water. Next, the level of the fluid in the filtration dish was lowered to a depth of 1 mm., or less if desired, by removing the necessary amount from the watch glass. Thus many hundreds of didinia could be concentrated without injury in a very small volume of fluid in the filtration dish. Finally, the didinia were removed in very little fluid from the dish by means of a small pipette, and were counted into the experimental slides as they left the pipette.

The experimental method employed in most of the encystment tests was relatively simple. Different numbers (1 to 300) of well-fed didinia were deprived of food in constant volumes (0.2 cc.) of fluid in the concavities of depression slides. The slides were kept in moist chambers at 22°C. The number of cysts was counted in each concavity, first at the end of 24 hours, and again at the end of 48 hours, reckoned from the beginning of the experiment. Very few cysts were produced prior to the 24th hour, and none after the 48th hour, so that earlier and later counts were not justified. The number of unencysted—hence, actively swimming—specimens was also counted at the end of 48 hours in order to get the total number of specimens (encysted and active) produced in the absence of food. From this total and from the number of encysted specimens present after 24 or 48 hours, the percentage of encystment for these respective hours was readily calculated. (Reference may be made to Beers, 1927, p. 503, for further explanatory comments on procedure.)

Although two clones were used in the study, the results obtained with each were to all practical purposes identical, and these results are combined in the accompanying tables without regard to clonal designation.

RESULTS OF TESTS IN DEPRESSION SLIDES

The results of the various experiments carried out in 0.2-cc. volumes of fluid in depression slides are summarized in Table 1. For convenience in description, the results will be considered as separate experiments.

Experiment 1.—In this experiment different numbers of didinia (1 to 300) were

deprived of food in 0.2-cc. volumes of natural spring water of pH 6.6. Although spring water is of variable chemical composition, its reaction and tonicity are generally favorable for *Didinium*, and hence it was selected as the first of the fluids to be tested in a study of the crowding effect.

An examination of Table 1 shows the following with reference to this experiment. Sixty didinia were deprived of food, each in 0.2 cc. spring water. They produced a total of 147 individuals in the absence of food (an average of 2.45 progeny each). Of these, none had encysted at the end of 24 hours and only 8.1 per cent of them (representing 12 individuals) at the end of 48 hours when the final count was made. Next, 100 didinia were deprived of food in groups of 5 per 0.2 cc. spring water. They produced 247 offspring, of which 24.3 per cent were encysted at the end of 24 hours and 49.4 per cent at the end of 48 hours. Then, 200 didinia were deprived of food in groups of 10 per 0.2 cc. Of their 495 progeny, 32.3 per cent were encysted after 24 hours and 44.4 per cent after 48 hours. The percentages obtained with groups of 5 or 10 didinia are of special interest in view of their close agreement with earlier results furnished by didinia collected in Baltimore, Maryland, and similarly deprived of food (Beers, 1927, 1927a). Next, 150 didinia were deprived of food in 3 groups of 50 specimens each. They produced 332 progeny, of which 70.2 per cent encysted within the first 24 hours and 93.4 per cent within the usual 48-hour period. Thus, didinia isolated in groups of 50 per 0.2 cc. showed a distinctly higher percentage of encystment, whether at the end of 24 or 48 hours, than individuals isolated singly or in groups of 5 or 10. Finally, 2 groups of 100 individuals each and 1 group of 300 individuals were isolated. The percentage of encystment at the end of 24 hours amounted to 88.0 and 97.4, respectively, and at the end of 48 hours it exceeded 98.

These results show clearly that in natural spring water the percentage of encystment increases markedly as the number of didinia per unit volume of fluid increases. Furthermore, the density of population needed to induce 98 per cent encystment is by no means so great as was anticipated when the experiments were begun. For example, an initial density of 100 didinia per 0.2 cc. amounts to only 1 individual per 2 cu. mm. Yet at this relatively low concentration of ciliates the encystment rate reached almost 90 per cent at the end of 24 hours and exceeded 98 per cent at the end of 48 hours. A higher initial concentration of animals (300 per 0.2 cc. or 1 per 0.67 cu. mm.) produced a higher percentage of encystment at the end of 24 hours, though but little higher than 98 per cent at the end of 48 hours. Hence, it is evident that an initial concentration of one didinium per 2 cu. mm. is adequate for the attainment of an encystment rate of approximately 98 per cent. Further experiments employing a variety of different fluids, the results of which will be presented shortly, will support this general conclusion. Actually, the final concentration is approximately twice the initial concentration, since each didinium divides at least once in the absence of food. Final concentrations may be calculated readily from the data of Table 1. Consider, for example, the fourth line of Experiment 1. The first item (50) divided into the second (150) shows that three groups (hence 0.6 cc. or 600 cu. mm. of fluid) are involved. Upon dividing 600 by 332 a final concentration of 1 didinium

to 1.8 cu. mm. is obtained. However, the experimental procedure may be related more readily to the final percentages if the initial concentration is emphasized.

TABLE 1

Effect on encystment of depriving different numbers of didinia of food in a constant volume of fluid (0.2 cc. in the concavity of a depression slide at 22°C.)

Note that as the initial number of didinia per concavity increased the percentage of encystment increased, regardless of the fluid employed.

EXPERIMENT NUMBER AND FLUID MEDIUM	NUMBER OF DIDINIA ISOLATED IN EACH CONCAVITY	TOTAL INITIAL NUMBER OF DIDINIA SO ISOLATED	TOTAL NUMBER OF DIDINIA PRODUCED IN ABSENCE OF FOOD	PERCENTAGE ENCYSTED AFTER 24 HOURS	PERCENTAGE ENCYSTED AFTER 48 HOURS
1	1	60	147	0.0	8.1
Natural Spring Water	5	100	247	24.3	49.4
	10	200	495	32.3	44.4
	50	150	332	70.2	93.4
	100	200	418	88.0	98.5
	300	300	608	97.4	98.3
2	1	248	618	0.0	7.1
Buffered Spring Water	5	400	865	26.6	43.9
pH 6.8	10	460	1,098	38.1	51.5
	20	320	678	46.6	60.8
	50	450	928	75.5	82.7
	100	500	1,051	94.9	97.5
	300	600	1,240	96.3	98.8
	500	500	1,030	97.8	99.1
3	1	66	171	0.0	7.0
Artificial Spring Water	5	200	458	17.0	39.3
	10	280	608	13.1	41.9
	50	200	438	64.4	85.8
	100	200	412	93.4	97.3
	300	300	639	95.5	98.7
4	1	100	258	1.9	6.2
Hay Infusion Culture of	5	300	708	10.6	39.8
Bacteria	10	400	998	38.2	53.4
	50	300	751	39.5	92.3
	100	400	892	93.1	97.8
	300	600	1,288	96.0	98.9
5	1	72	201	0.0	0.0
Excretion Products of	5	190	529	0.0	8.1
Paramecium	10	100	226	16.7	50.3
	50	100	208	88.4	93.2
	100	100	206	94.1	98.0
	300	300	627	97.6	98.5

The findings indicate further that the high percentages of encystment result from the higher concentration of didinia, or more precisely from a higher concentration of their metabolic products. To this thesis it may be objected: (1) that

changes in the pH of the medium when large numbers of didinia are present are the responsible factors; (2) that the number of bacteria increases as the metabolic by-products accumulate and that the bacteria account for encystment; and (3) that the supply of oxygen is depleted as the number of didinia increases, thus inducing encystment through oxygen deficiency. These objections will be considered in turn.

As to changes in the hydrogen ion concentration of the medium, it is true that the pH increased slightly (from 6.6 to 7.0–7.4) in all the test cultures, the slides themselves being made of soft glass, and that it showed the greater increase in cultures containing larger numbers of didinia. Since some investigators, among them Koffman (1924) and Darby (1929), attribute considerable importance to pH changes as factors inducing encystment, the foregoing experiments were repeated in buffered spring water. The results follow immediately.

Experiment 2.—In this experiment natural spring water was buffered with Clark and Lubs' KH_2PO_4-NaOH buffer mixture, prepared according to Clark (1928), in the proportion of 5 cc. buffer mixture of pH 6.8 to 100 cc. spring water. Actually, this amount of buffer mixture was more than adequate to keep the pH constant for the duration of the experiment. In plan the experiment was essentially like Experiment 1, though 16 groups of 20 didinia each and one group of 500 didinia were included. On the whole, the results (Table 1) were in close agreement with those of Experiment 1. As the initial number of didinia increased, the percentage of encystment increased, both at the end of 24 hours and 48 hours. The groups of 20 didinia were more or less intermediate with reference to encystment rate when compared with groups of 10 or 50. The 5 groups of 100 didinia each showed a slightly, though insignificantly, lower final percentage of encystment than the corresponding groups of Experiment 1. The 2 groups of 300 individuals agreed closely with those of the preceding experiment. The single group of 500 didinia yielded almost the same final percentage of encystment as the groups of 300. A few didinia seemed to be consistently refractory throughout the experiments, so that an encystment rate of 100 per cent was never realized when totals were computed.

In conclusion, the results of Experiment 2, in all parts of which the pH remained unchanged, show that the higher percentage of encystment in the crowded experimental cultures may not be attributed to changes in hydrogen ion concentration, but is in all probability an effect of the higher concentration of animals.

Experiment 3.—Since the chemical components of natural spring water are extremely variable and include not only simple inorganic salts but organic compounds of wide variety and complexity, a test of the crowding effect in an inorganic medium of known composition seemed advisable as a means of eliminating undefined variables. Therefore, the effect was tested, in accordance with the plan of Experiments 1 and 2, in the following medium, which for convenience will be referred to as artificial spring water:

0.1% Na_2SiO_3	4	cc.
0.1% NaCl	4	cc.
0.1% Na_2SO_4	2	cc.

0.1% $CaCl_2$	2 cc.
0.1% $MgSO_4 \cdot 7H_2O$	2 cc.
0.1% $FeCl_3 \cdot 6H_2O$	1 cc.
Distilled water	85 cc.
KH_2PO_4-NaOH buffer mixture of pH 7.0	2.5 cc.

This medium may be regarded as a modification of the "artificial spring water" recommended by Hopkins and Pace (1937, p. 78) for the culture of *Amoeba proteus*. The modification is largely empirical. Didinia when transferred into it from spring water-paramecia cultures show no abnormalities of structure or behavior; they exhibit no spinning on a transverse axis, no reversal of direction, and no erratic, jerky spiraling. Hence, the medium appears to be entirely favorable for *Didinium*.

The results of this experiment require no special comment, in view of their close agreement with those of Experiments 1 and 2. Reference to Table 1 shows that the usual increase in the percentage of encystment occurred as the initial number of didinia was increased. Since the medium was adequately buffered, changes in pH were in no wise responsible for the results. Finally, since the medium itself was wholly unsuited to the growth of ordinary bacteria and contained after 48 hours only insignificant numbers of them, as revealed by microscopic examination, it is evident that bacteria were in no way related to the encystment process. Hence, the conclusion is inescapable that the high percentages of encystment can be accounted for only in terms of the higher density of population.

The cysts produced in artificial spring water were entirely normal. Samples of them, when tested for excysting capacity by immersion in 0.5 per cent bacterized peptone solution (Beers, 1945, 1946), showed 80–90 per cent excystment.

Experiment 4.—It is well known that *Didinium* cysts can be activated regularly by immersion in a rich hay-infusion culture of suitable bacteria, such as is often employed for the growth of *Paramecium* (Mast, 1917; Beers, 1937). The paramecia themselves are not necessary for excystment. Indeed, the findings of Beers (1945, 1946) show with reasonable conclusiveness that the bacteria which occur commonly in organic media (hay and lettuce infusions or peptone and yeast-extract solutions, for example) are the agents which are primarily responsible for excystment in *Didinium*. Since these bacteria are highly effective excysting agents, the question naturally arises as to whether they are also capable of preventing or retarding encystment, thereby offsetting the usual effects of crowding as set forth in Experiments 1–3. This point is tested in the present experiment.

Fresh timothy-hay infusion (about 2 gm. chopped, dry timothy to 100 cc. water) was heavily inoculated with wild bacteria from a flourishing *Paramecium* culture. At the end of a week, when the bacterial activity was intense and the pH of the infusion was about 7.4, the infusion was tested for excystment-inducing capacity and found to be highly effective. This infusion with contained bacteria was used as the fluid medium in the encystment tests of Experiment 4, which in their execution were identical in plan with those of Experiments 1–3.

When the tests of Experiment 4 were begun, it was fully expected that such a rich bacterial infusion would retard encystment. In reality, this expectation was

in no wise realized, since the results (Table 1) were in complete accord with those of Experiments 1–3. An increase in the initial number of didinia per 0.2 cc. infusion produced the usual increase in the percentage of encystment. Thus the metabolites of *Didinium*, which are assuredly present in greater concentration in the denser test cultures and which presumably account for the higher percentages of encystment, are sufficiently stable to be effective even in the complex of organic compounds which constitutes a hay-infusion culture.

Experiment 5.—The writer (1927) presented evidence indicating that the metabolic by-products of *Paramecium* inhibit the encystment of *Didinium* in the absence of food. Large numbers of paramecia were washed free of hay infusion and were concentrated in a few cc. of spring water, in which they remained 3 days to permit their metabolites to accumulate. The paramecia were then removed by filtration, and the encystment of groups of 5 didinia per 0.2 cc. filtrate was tested. Almost none of the didinia encysted, though in the spring water controls they showed the usual encystment rate of about 50 per cent. Groups of more than 5 individuals were not tested.

In Experiment 5 of the present study, the former experiment was repeated, and also extended to include single individuals and groups of 10, 50, 100 and 300. The paramecia were washed and concentrated as formerly (Beers, 1927, p. 511), though *P. multimicronucleatum* was used instead of *P. caudatum*. Their concentration in the spring water in which their metabolites were allowed to accumulate amounted to about 50,000 per cc. After 3 days they were removed by centrifuging, and the various groups of didinia were transferred immediately to 0.2-cc. amounts of the supernatant.

The results obtained with didinia isolated singly or in groups of 5 (Table 1) were in general agreement with the earlier results. None of the 72 specimens which were isolated singly encysted, whereas 6 to 8 per cent of them encysted in Experiments 1–4. As to the groups of 5 didinia each, none encysted within the first 24 hours and only 8.1 per cent after 48 hours. These percentages were well below the corresponding percentages of Experiments 1–4. Thus the present results with didinia isolated singly or in groups of 5 indicate again that the metabolites of *Paramecium*, whether *P. caudatum* or *P. multimicronucleatum*, inhibit the encystment of *Didinium* in the absence of food. However, an examination of the results obtained with groups of 10, 50, 100 or 300 didinia reveals no evidence of an encystment-inhibiting effect. The percentages of encystment obtained with these groups were not significantly different from those obtained with the corresponding groups of Experiments 1–4. Thus the effect of the substances produced by *Paramecium* appears to be readily counteracted when the initial concentration of the didinia is increased to 10 or more per 0.2 cc. of fluid. The results serve to emphasize the high potency or effectiveness of the encystment-inducing substance (or substances) elaborated by *Didinium*.

RESULTS OF TESTS IN GLASS TUBING

In criticism of the foregoing results it may be objected, as was mentioned earlier, that the higher percentage of encystment in the crowded test cultures was induced by the depletion of the amount of dissolved oxygen and that the

animals therefore encysted because of oxygen deficiency. Actually, this explanation seems improbable in view of the large surface and shallow depth of 0.2 cc. of fluid in a concavity which measures 18 mm. in diameter and 1.8 mm. in maximum depth. Nevertheless, further studies were made to test this possibility. In these studies the amount of oxygen available to the didinia was drastically limited, and the tests were designed to show whether such limitation induces encystment more readily (in less than 24 hours) or results in a higher percentage of encystment at lower densities of population.

In these tests pieces of glass tubing having a length of 10 mm. and an inside diameter of 2 mm. were used. One end was sealed by flaming, so that each piece was converted into a miniature test tube. In use, each tube contained a column of spring water 3 mm. in length—a total volume of approximately 10 cu. mm. of spring water. Different numbers of didinia were put into these tubes by means of a small pipette and their history in the absence of food was followed. The tedious nature of the work placed a practical limitation on the number of tests made.

The following three categories of tests were made: (1) The tubes, containing different numbers of didinia, were left unsealed at the upper end and were kept vertically in a low sealed Stender dish which contained some water and served as a moist chamber. These tubes permitted comparisons with the depression-slide tests and served as controls for the tests of the second category. (2) The tubes, again containing different numbers of didinia, were sealed at the top by means of a 3-mm. layer of moderately heavy paraffin oil which was in direct contact with the meniscus of the spring water. Thus the amount of oxygen available to the animals was limited to the amount already in solution in the spring water. If oxygen deficiency hastens the onset of encystment, the didinia in the tubes of high population density should encyst in less than 24 hours and should show a high percentage of encystment. (3) The paraffin oil was put into the tube first, and the spring water and didinia were put in next, with the spring water in contact with the paraffin oil. This procedure is feasible, if the paraffin oil is sufficiently heavy and the diameter of the tube is sufficiently small, but such tubes must not be tilted unduly. These tubes were necessary as a second control to tell whether or not the paraffin oil was exercising an effect. If the paraffin oil were quite inert, the results should agree with those of the tubes of the first category.

Some of the encystment tubes were prepared in the morning and examined after 12 hours; usually they were prepared in the afternoon and examined first at the end of 18 hours. None of the 12- or 18-hour examinations revealed any mature cysts. Hence, none of the experiments conducted in tubes reduced the amount of time required for encystment. The results of these tests are therefore presented in Table 2 quite as the earlier results were presented in Table 1.

The counts made on tubes at the end of 24 hours are not absolutely accurate with reference to numbers of encysted specimens, though they are reasonably close approximations. These counts were made under the dissecting binocular by holding the tube at an angle and rotating it slowly. Under these optical con-

ditions an unencysted specimen at rest is not always readily distinguished from a cyst. The final counts offered less difficulty, since the tubes were first emptied into a depression slide; the cysts remained attached to the inner wall of the tube the active specimens were found in the fluid.

Experiment 6. Tubes open at the top.—At first sight some of the results of this experiment appear to be in disagreement with those of Experiments 1–4. Actually this is not the case. For example, didinia isolated singly in Experiment 6 showed an encystment rate of 21.6 per cent after 24 hours and 56.9 per cent after 48 hours (Table 2), whereas didinia isolated singly in Experiments 1–4 showed percentages varying from 0.0 to 8.1. However, in Experiments 1–4 each single didinium was isolated in 0.2 cc., but in Experiment 6 each was isolated in 10 cu. mm.; this concentration is equivalent to the 20 animals per 0.2 cc. of Experiment 2, in which 60.8 per cent encystment was attained after 48 hours. Thus a final percentage of 56.9 in Experiment 6 is in fairly close agreement with the corresponding percentage (60.8) of Experiment 2. A concentration of 5 didinia per 10 cu. mm. (Experiment 6) is equivalent to 100 per 0.2 cc. (Experiments 1–4) or 1: 2 cu. mm. It has already been pointed out that this concentration may be depended on to induce approximately 98 per cent encystment at the end of 48 hours. In Experiment 6 the percentage was 97.0. A concentration of 10 per 10 cu. mm. gave similar results, and a concentration of 50 per 10 cu. mm. (1: 0.2 cu. mm.—the highest tested) failed to increase the final percentage of encystment. Thus it seems to be impossible to attain 100 per cent encystment consistently in *Didinium*. Certain tubes or slides may show 100 per cent; but other similar tubes or slides will contain a few active specimens and thus reduce the total percentage. On the whole the tests made in tubes confirm the findings yielded by slides, when equivalent initial concentrations of animals are compared, and show again that the encystment rate in the absence of food varies directly with the density of population.

Experiment 7. Tubes sealed with paraffin oil to exclude oxygen.—Didinia isolated singly showed an encystment rate of 48.0 per cent, in general agreement with those of Experiment 6 (56.9 per cent) and in fair agreement (60.8 per cent) with those of the corresponding concentration (20: 0.2 cc.) of Experiment 2. Didinia isolated in groups of 5 (1: 2 cu. mm.) showed slightly lower percentages of encystment (28.2 and 91.6) than those of corresponding concentrations (100: 0.2 cc.) of Experiments 1–4. Higher initial concentrations (10 or 15: 10 cu. mm.) showed still lower final percentages of encystment (86.3 and 87.5), which, as will be seen shortly, were to be correlated with the exclusion of oxygen. In the next concentration to be considered, 20 didinia per 10 cu. mm., the effect of lack of oxygen began to express itself markedly, though not in an increase in encystment rate or in the earlier production of cysts. At the end of 24 hours there were no cysts in the 3 tubes, and the active specimens (about 126) were swimming sluggishly. At the end of 48 hours the encystment rate amounted to only 3.2 per cent, representing 4 cysts in 3 tubes, and the unencysted specimens were dead and disintegrating at the bottom of their respective tubes. The 4 cysts disintegrated on the following day. Thus exclusion of oxygen at an initial concentration

of 20 didinia per 10 cu. mm. of fluid actually prevented encystment. Therefore, encystment does not serve to protect the animals against depletion of the oxygen supply. A single tube containing 50 didinia remains to be considered. Most of these didinia failed to divide. At the end of 24 hours not more than 60 sluggishly swimming individuals were present in the tube and there were no cysts. Twenty-four hours later the didinia were dead, and with no visible attempts at cyst production.

The results of this experiment show that a reduction in the oxygen supply does not lead to encystment. If sufficient oxygen is present to sustain life, the didinia

TABLE 2

Effect on encystment of depriving different numbers of didinia of food in miniature glass test tubes, each containing 10 cubic millimeters of natural spring water. Temperature, 22°C.

EXPERIMENT NUMBER AND CHARACTER OF TUBES	NUMBER OF DIDINIA PUT INTO EACH TUBE	TOTAL INITIAL NUMBER OF DIDINIA SO TREATED	TOTAL NUMBER OF DIDINIA PRODUCED IN ABSENCE OF FOOD	PERCENTAGE ENCYSTED AFTER 24 HOURS	PERCENTAGE ENCYSTED AFTER 48 HOURS
6	1	20	51	21.6	56.9
Tubes Unsealed	5	25	64	84.4	97.0
	10	40	89	91.0	98.8
	50	50	111	91.9	97.3
7	1	20	46	13.0	48.0
Tubes Sealed with	5	60	132	28.2	91.6
Paraffin Oil	10	80	188	25.5	86.3
	15	30	64	28.1	87.5
	20	60	ca. 126	0.0	3.2
	50	50	ca. 60	0.0	0.0
8	1	10	27	14.8	44.4
Oil below, Water above	5	20	44	63.6	95.5
	10	40	89	88.1	98.5
	20	50	109	87.8	96.4

encyst, provided the density of population is sufficiently high. If the amount of oxygen is inadequate to sustain life, the didinia die and thus produce no cysts.

Although it is true that the procedure adopted in Experiment 7 not only excludes oxygen but permits the carbon dioxide produced by the animals to accumulate in the medium, it seems improbable that any of the results may be attributed to carbon dioxide in excess. After making the final counts in this experiment, the pH of the small amounts of fluid was determined colorimetrically, as best it could be done. Comparisons with standard buffer mixtures showed that it varied from 7.0 to 7.4. An excessive amount of carbon dioxide in solution should result in a much lower pH value. Hence, the evidence, though not absolutely conclusive, indicates strongly that carbon dioxide in excess does not account for the failure of the didinia to encyst.

Experiment 8. Oil at bottom of tube, didinia in spring water on oil.—As has

been pointed out, this experiment was needed as an accompaniment of Experiment 7 to tell whether the paraffin oil might be held in any way responsible for the results of Experiment 7. If the oil is without effect, the results should agree with those of Experiment 6, since the tubes of this experiment were likewise unsealed at the top. An examination of Table 2 shows that the final percentages of encystment agreed satisfactorily with those of Experiment 6. Hence, none of the results of Experiment 7 may be attributed to the contact of the paraffin oil with the water.

ENCYSTMENT IN THE PRESENCE OF FOOD

The encystment of *Didinium* in the presence of ample food has been observed by a number of investigators, among them Mast (1917), Mast and Ibara (1923), Reukauf (1930), and Rammelmeyer (1931). That such encystment is actually the result of excessive crowding of the didinia was readily demonstrated in the present study by preparing small mass cultures and following their history. The preparation and the usual history of each such culture follow.

Twenty didinia and several thousand paramecia, which had been washed and concentrated in spring water, were put into a Columbia culture dish. Sufficient spring water was added to bring the volume up to 1 cc., and the dish was set aside in a moist chamber at 22°C. After 24 hours such a dish usually contains about 300 didinia, and after 36 hours (counting from the beginning), about 1200. A few hours later, when the number of didinia in such a culture reaches about 1500, encystment regularly sets in, even though many hundreds of paramecia are still present. (Indeed, paramecia must still be present, otherwise the experiment is a failure. The number of paramecia to be added originally is best determined by experience; the dietary needs of *Didinium* are incredible and the number must be great.) After 48 hours the culture usually contains 1200–1500 cysts and 100–300 active, feeding specimens.

Thus didinia may be induced to encyst in the presence of food, provided a sufficiently high density of population is reached. However, the percentage of encystment in these cultures is never as high, in my experience, as in cultures in which the factors absence of food and crowding are operating simultaneously.

DISCUSSION

The results of the present study show that in *Didinium* the absence of food must be regarded as little more than a weak encystment-inducing stimulus and as inadequate to produce the encystment of more than 8 per cent of the animals. These conclusions are brought out by the isolation of single didinia in a variety of fluid media. As in *Colpoda cucullus*, absence of food must therefore be looked on as a factor of secondary importance in inducing encystment in *Didinium*. In the test cultures, and in all probability in nature, the tendency of a single didinium, it seems, is to continue to swim and to search for food. Actually, the food of *Didinium* is obtained intermittently and always in the form of large portions which usually can be located only by diligent search. A too ready ten-

dency to encyst in the absence of food might conceivably militate against the survival of the species, since there is no reproduction within the cysts and since the cysts themselves are subjected in nature to a variety of hazards, including ingestion by hypotrichs and other aquatic animals, and the vagaries of water currents and weather. Thus each didinium perseveres diligently in the search for food, and should it encyst, it does so only after a lengthy search covering a period of at least 24 hours.

Crowding or the attainment of a high density of population appears to be the factor of primary importance in inducing encystment. Thus encystment occurs even when food is still present, provided the density of population is high enough. When crowding is combined with absence of food, the encystment rate reaches almost 100 per cent. There can be no doubt that this combination of conditions, whether in the laboratory or in nature, is highly unfavorable for the survival of didinia in the active state; each such didinium, even in nature, faces little more than a remote possibility of finding food. Hence, the animals, under conditions involving crowding and lack of food, encyst readily. In view of the present experimental evidence, the conclusion is unavoidable that such encystment is induced primarily by substances which the didinia themselves produce. The evidence indicates, furthermore, that the didinia are very sensitive to these substances, since the actual concentration of animals need not be excessively high to induce a high percentage of encystment.

SUMMARY

Different numbers of didinia, usually from 1 to 300, were deprived of food in constant volumes (0.2 cc.) of different fluids, among them, natural spring water, buffered spring water, artificial spring water, and vigorous hay-infusion culture of wild bacteria. These fluids gave uniform results. Single didinia showed a very low encystment rate, never exceeding 8 per cent. As the initial number of didinia was increased (5, 10, 50, 100 and 300 per 0.2 cc.), the percentage of encystment increased. Initial population densities of 100 and 300 didinia per 0.2 cc. induced 98 per cent encystment.

Experiments conducted with smaller volumes of fluid (10 cu. mm.) in miniature test tubes confirmed the foregoing results. The exclusion of atmospheric oxygen by sealing some of the tubes with paraffin oil failed to hasten the onset of encystment or to increase the final percentage of encystment. The absence of an adequate supply of oxygen led only to the death of the didinia.

The excretion products of *Paramecium* inhibited encystment in the absence of food when the concentration of didinia was low (1 or 5 per 0.2 cc.). They were without effect at higher densities of population.

Didinia were induced to encyst readily in the presence of food by limiting the volume of fluid and thus allowing the density of population to increase.

The results indicate that absence of food is a factor of secondary importance in inducing the encystment of didinia. The accumulation of their own metabolic waste appears to be primarily responsible for encystment, no matter whether food is present or absent. A combination of absence of food and a high density

of population (with consequent accumulation of waste) is highly effective in inducing encystment.

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A STUDY OF SOME ALGAL PIGMENTS

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Three Text Figures

Color, or characteristic pigmentation, is one of the criteria used in the separation of the classes of algae. The idea long held by workers in the algae, that each of the classes has a characteristic set of pigments, has recently been amply justified for several of the classes (Smith, 1933, 1938; Fritsch, 1935, 1945). The most recently recognized class of algae, the Chrysophyceae, is composed for the most part of single-celled plants or microscopic colonies rarely collected in abundance. Consequently, the pigments of this class have been little studied. A recently described species (*Phaeosphaera perforata*) belonging to this class (Whitford, 1943) forms large colonies. A sufficient quantity of material has been collected to make possible a study of some of its pigments. This paper is a brief report of the results of this investigation.

Klebs in 1892, working with certain golden brown flagellates, reported a pigment characteristic of the group and named it chrysochrome. Gaidukov (1900) renamed the pigment phycochrysin after he had isolated it from a species of *Chromulina*. During the next forty years almost no work was done on the pigments of this group which, among others, is now included in the Chrysophyceae. According to Fritsch (1935) little is yet known about phycochrysin. Smith (1933, 1938), however, expressed doubt as to the existence of a special pigment in the Chrysophyceae and Bacillariophyceae.

Meanwhile work was being done on the pigments of other groups. The chief accessory pigment of the brown algae, fucoxanthin, received considerable attention and Willstätter and Page published its probable formula in 1914. It is only recently, however, that the pigments of smaller forms have been investigated by means of chromatographic adsorption analysis—a method uniquely adapted to the study of pigmented compounds. Heilbron and Phipers (1935), using this method, studied the pigments of green, red, and brown algae. In 1940 Carter, Heilbron, and Lythgoe compared the lipochromes of seven classes of algae. Included were three species in the Chrysomonadales. They reported the presence of fucoxanthin in twelve species of Phaeophyceae, three species of Chrysophyceae, and one species of Bacillariophyceae. They also found carotene and another xanthophyll (lutein) in all sixteen species studied. Seybold and Egle (1938) also reported fucoxanthin in diatoms as well as brown algae. Pace (1940), on the other hand, did not find fucoxanthin in the diatom *Nitzschia closterium* but reported lutein, isolutein, and cryptoxanthin in addition to chlorophyll *a* and *b* and carotene. Strain and Manning (1942, 1943) made contributions to our knowledge of chlorophylls and accessory pigments of the brown algae and diatoms. They point out that isomers of xanthophylls occur naturally in plants and also may

form during extraction and handling. Strain, Manning and Hardin (1944) report several new xanthophylls from diatoms, brown algae, and dinoflagellates. They again found fucoxanthin in diatoms and brown algae but did not find it in dinoflagellates nor the one species of Xanthophyceae (*Tribonema bombycinum*) studied. In determining the homogeneity of pigments they suggest the use of several adsorbents and solvents. They also point out that the solvent may have a pronounced effect on the spectrum adsorption curve of pigments.

A good summary of our present knowledge of plant pigments is given by Rabinowitch (1945).

METHODS AND RESULTS

A number of extraction methods were tried before a fairly satisfactory method was found to separate *Phaeosphaera* pigments. Since the cells are scattered in a soft pectic mass containing a large amount of water, 95–100% ethyl alcohol was added to the mass so that the actual concentration that reached the cells was approximately 80%. The carotinoid pigments first streamed out rapidly, leaving the cells blue-green in color. In about 20 minutes, however, practically all the chlorophyll also had dissolved. The resulting solution was filtered into a separatory funnel and about $\frac{1}{3}$ to $\frac{1}{2}$ the volume of petrol ether added. Water equal to $\frac{1}{2}$ the total volume was then added and the funnel rotated to allow the pigments to dissolve in the petrol ether. The chlorophyll and most of the carotinoid pigments dissolved in the petrol ether. The lower, light yellow, alcohol-water layer was drawn off and saved for further extraction. Sometimes a foamy mass forms and interferes with the separation. A small amount of an inorganic salt (such as sodium sulfate) overcomes this difficulty. The petrol ether solution was washed several times to remove all remaining alcohol. It was then drawn off into a small flask and anhydrous sodium sulfate added to remove the last traces of water. Enough was added so that considerable residue of the anhydrous powder remained. The dried pigment solution was then ready for chromatographic analysis.

Some of the carotinoid pigments are not very soluble in petrol ether; therefore, ethyl ether was now added to the alcohol-water extract. The remaining pigments dissolved in the ether leaving the alcohol-water layer colorless. This ether extract was evaporated and the pigments were re-dissolved in petrol ether. The resulting solution was added to the first (petrol ether) extraction.

The adsorption tubes used were of glass, and were 15–20 times as long as the inside diameter. Some were drawn out at the lower end so that a rubber suction tube could be attached. If the column was to be sectioned a tube of uniform diameter was used with a 1-hole rubber stopper and a small suction tube at the lower end. The most satisfactory adsorptive column was found to be the Lintner soluble starch column suggested by Curtis (1942). Several brands of starch formed equally satisfactory columns.

The starch was made into a slurry of about the consistency of table syrup with petrol ether, and completely freed of lumps. A pledget of cotton was placed in the bottom of the adsorption tube to support the column and the starch slurry

was poured rapidly in until the column was nearly or quite full. The starch soon settled leaving a layer of petrol-ether on top. A small suction flask was attached to the bottom of the tube and suction applied with an ordinary filter pump. The tube was tapped as the starch continued to settle to prevent the formation of cracks or bubbles. A uniform column of starch was usually obtained by the time the petrol ether layer reached the surface of the settled starch. At this time an amount of the concentrated pigment solution equal to about $\frac{1}{10}$ the volume of the starch column was poured in. If the column was "overloaded" a satisfactory chromatograph was not obtained.

As the pigment moved into the starch column it was adsorbed in a wide yellow-green band at the top of the column. As the last of the solution entered the starch column pure dry petrol ether was added to the tube. As suction continued several pigments separated from the green layer and moved down the column. An orange pigment (carotene) moved rapidly through the column and collected in the suction flask. A narrow clear yellow band (xanthophyll No. 2) moved less rapidly, a wider brownish-orange band (xanthophyll No. 1) moved still less rapidly, and the chlorophyll moved most slowly of all. If 200–300 cc. of petrol ether were drawn through a column (20 mm. in diameter), the chlorophyll moved down several centimeters, the brownish-orange band was just below and usually touching the chlorophyll band, and about one centimeter below this was the clear yellow band. If a benzene-petrol ether mixture (1:14) was carefully added a somewhat more rapid movement of the bands occurred and the brownish orange band could usually be separated from the chlorophyll band (Fig. 1).

When good separation of the bands was achieved the lower stopper was removed and the moist column pushed out on a sheet of paper. Each band was sectioned from the column. Where two bands touched a narrow section between them was discarded. Each of the pigments was eluted from the starch with ethyl ether, the solvent evaporated, and the pigment re-dissolved in petrol ether.

Because of its color and abundance the brownish-orange pigment was assumed to be the accessory pigment giving the plant its characteristic golden brown color. As mentioned above, the accessory pigment in the Chrysophyceae was first called chrysochrome or phycochrysin but recent workers have reported it to be fucoxanthin. Since chromatographic work has shown that identical pigments cannot be separated on chromatographic columns, but that different pigments and even isomers can be separated, it was decided to compare this pigment with the similar pigment in a brown alga and a diatom. Fresh material of *Fucus vesiculosus*[1] and *Phaeosphaera* were extracted by the same methods and the pigments separated on two starch columns. The chief accessory pigment was identical in color and position in the two columns. These brownish-orange bands were sectioned from the columns, eluted with ethyl ether, evaporated, re-dissolved in petrol ether, and divided each into two portions. Three starch columns were prepared at the same time. Half the *Phaeosphaera* pigment was drawn through one column, half the *Fucus* pigment through another and the two remaining

[1] The *Fucus* was collected at the Duke Marine Laboratory, Beaufort, N. C., by Dr. H. J. Humm.

portions were mixed and drawn through the third column. In each tube to which a single pigment had been added, a single brownish-orange band formed at the same level. In the tube with the two pigments a somewhat wider band formed at the same level as in the two other tubes. All three bands were drawn down 4 cm. below where they were first adsorbed. In no case was there any indication of a double band. This is strong evidence that the selected pigments in *Fucus*

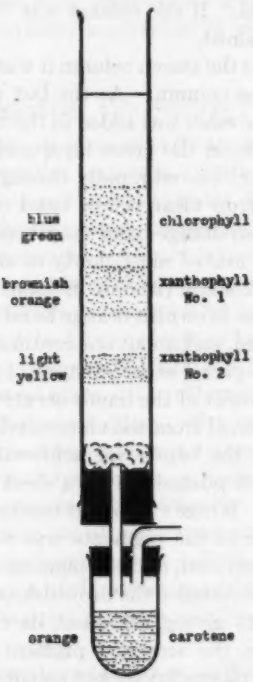

FIG. 1. Chromatograph of *Phaeosphaera* pigments on starch column. Solvent petrol ether. Developed for 20 min. with benzene-petrol ether (1:14). Color of band at left, name of pigment at right.

and *Phaeosphaera* are identical. Several repetitions always gave the same results (Fig. 2).

An unidentified species of diatom in the genus *Melosira* is quite abundant in the beds of streams near Raleigh in late winter. A quantity of this material was collected and the pigments extracted. The same brownish orange band formed just below the chlorophyll band when the pigments were adsorbed on a starch column. This pigment was compared with the *Phaeosphaera* pigment in the same manner as was the *Fucus* pigment. The same evidence was obtained that these pigments also are identical.

CHARACTERISTICS OF THE CHIEF ACCESSORY PIGMENT OF PHAEOSPHAERA PERFORATA

In living cells the pigment appears to be golden-brown in color. In solution (alcohol, ethyl ether, or petrol ether) it is a clear golden yellow. On the starch column it is brownish orange, and dried on porcelain it is dark brown in color. It is soluble in ethyl or methyl alcohol, in ethyl ether and acetone. It is somewhat less soluble in petrol ether and is insoluble in water. When living cells are killed in formaldehyde or hot water the pigment does not dissolve. Hot water

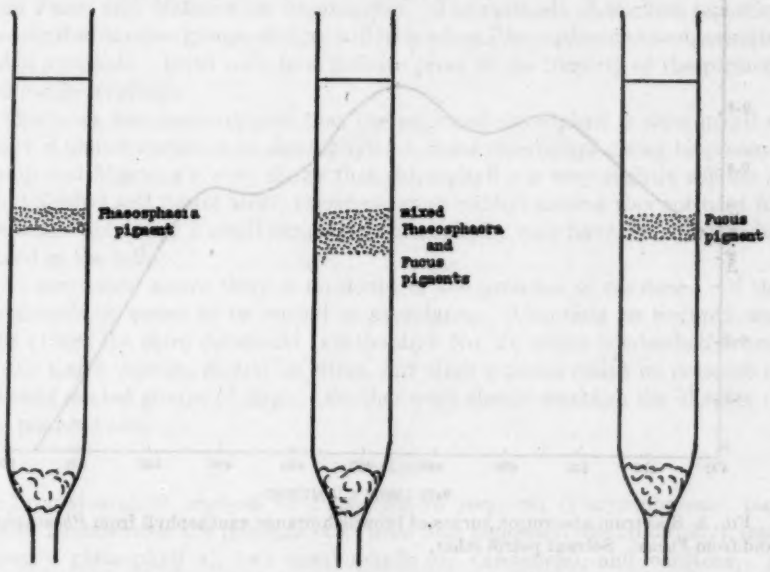

FIG. 2. Diagram of single and mixed chromatographs showing behavior of brownish-orange pigment from *Phaeosphaera* (xanthophyll No. 1) and *Fucus* on starch columns. A single homogenous band formed in each case.

seems to bring about a partial destruction of the carotinoid pigments, however, so that cells killed in hot water are greener than living cells.

Spectrum absorption curves were made of the pure pigment dissolved in petrol ether (Fig. 3). There are three maxima. The greatest transmittance is at 446 mμ; there is a smaller maximum at 424 mμ, and a still smaller one at 474 mμ. The absorption curve of the similar pigment from *F. vesiculosus* is identical with that from *Phaeosphaera*.

THE OTHER PIGMENTS

Little work has been done on the other *Phaeosphaera* pigments. From their behavior on adsorption columns, however, the following conclusions are justified. The orange pigment which is not adsorbed but passes rapidly through the starch

column is carotene (Curtis, 1942). The yellow band which is adsorbed below the brownish orange band is undoubtedly another xanthophyll (carotenol).[2] The highest (blue-green) band is chlorophyll. This closely resembles chlorophyll *a* from leaves when adsorbed on a starch column. Leaf chlorophyll separates on a starch column in two fairly definite bands, the yellowish green chlorophyll *b* above and bluish-green chlorophyll *a* below. Only one green band could be separated from the *Phaeosphaera* pigments. Chlorophyll *b* cannot be present unless in very small quantity.

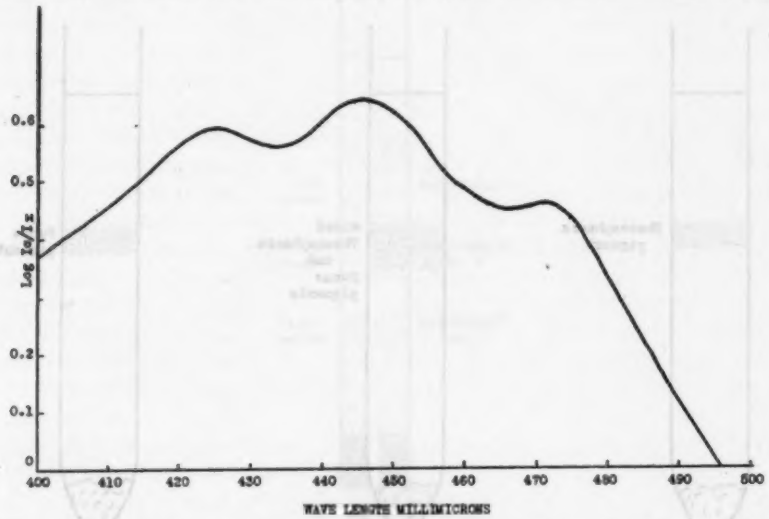

FIG. 3. Spectrum absorption curves of brownish-orange xanthophyll from *Phaeosphaera* and from *Fucus*. Solvent petrol ether.

DISCUSSION

The results of the experiments described above definitely indicate that the most abundant accessory pigment (carotinoid) in *Phaeosphaera perforata* (Chrysophyceae), *Fucus vesiculosus*, and *Melosira* sp. are identical. Comparison on both single and mixed chromatographs leaves little doubt, and the identical spectrum absorption curves of pigments from two of them (*Phaeosphaera* and *Fucus*) add further evidence of identical chemical composition. Similarities in color and solubility point to the same conclusion. As to the identity of the pigment there is some question. Most other investigators have reported fucoxanthin as the carotinoid characteristic of the Chrysophyceae, Phaeophyceae and Bacillariophyceae. The absorption curves of pigments extracted during this investigation,

[2] Bogert (1938) and Rabinowitch (1945) suggest that the term *carotenol* be used as a generic term, in place of xanthophyll, for the group of compounds which are usually hydroxyl derivatives of carotene, and the ending *-ol* rather than *-in* for individual compounds (e.g. fucoxanthol not fucoxanthin).

however, are not identical with that of fucoxanthin as reported by other workers. They are, in fact, somewhat similar to that of isolutein. On the other hand, as was pointed out above, the solvent used may have a pronounced effect on the shape of an absorption curve and even on the position of the maxima. The curves given here (Fig. 3) were made from a petrol ether solution and not the usual ethanol solution. In view of the amount of work that has been done on *Fucus* pigments there seems little doubt that its principal carotinoid is fucoxanthin. It seems likely therefore that the *Phaeosphaera* pigment and those isolated from *Fucus* and *Melosira* are fucoxanthin. The methods of workers reporting fucoxanthin in other groups of algae will be tried on *Phaeosphaera* as soon as material is available. Until such time definite proof of the identity of the pigment will not be available.

This work has demonstrated that the principal chlorophyll is chlorophyll *a*. There is almost certainly no chlorophyll *b*. Some chlorophyll *c* may be present. Strain and Manning's work shows that chlorophyll *c* is very slightly soluble in ethyl alcohol and petrol ether, therefore, since methyl alcohol was not used for the initial extraction a small amount of chlorophyll *c* may have remained undissolved in the cells.

As mentioned above there is no doubt of the presence of carotene. Of the carotinoids, it seems to be second in abundance. According to Seybold and Egle (1938) the third carotinoid (xanthophyll No. 2), which is adsorbed lowest on the starch column, should be lutein, but other workers doubt its presence in this and related groups of algae. Further work should establish the identity of this pigment also.

SUMMARY

Chromatographic analysis of *Phaeosphaera perforata* (Chrysophyceae) pigments demonstrates the presence of at least four pigments; one chlorophyll (apparently chlorophyll *a*), two xanthophylls (or carotenols), and carotene. A comparison of the most abundant brownish orange xanthophyll with similar pigments from a brown alga and a diatom by means of single and mixed chromatographs indicates that they are identical. The spectrum absorption curves of two of them also are identical. These curves are similar to, but not identical with those reported by other workers for fucoxanthin. The difference may be ascribed to the character of solvent (petrol ether) used when the absorption curves were made and, in some measure, to methods of extraction.

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MASS DISSEMINATION OF PINE POLLEN

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Two Text Figures

INTRODUCTION

In the dissemination of pine pollen what proportion shed by the trees falls directly to the ground and what proportion is carried to various distances from the trees? This paper, dealing with mass dissemination of pine pollen over short distances, gives a partial answer to this question. The investigation was stimulated by an uncertainty that arose during the course of a study of the fossilization of pollen of *Pinus serotina* Michx. (Buell, 1946). In that study surface samples of peat were used which were collected at the center of a large bog occupied by an open growth of the pond pine. The center of the bog was more than a quarter of a mile from the margin in any direction and hence to the nearest point where any other species of pine was growing. Entirely surrounding the bog on the upland was a forest of *Pinus palustris* Mill. It was assumed that a very large proportion, probably 90% or more, of the pollen grains in the surface samples were derived from *Pinus serotina* with only a very minor proportion of the pollen coming from the long-leaf pine. There was, however, no direct evidence as to the percentage of contamination from the surrounding forests. The best experimental evidence as to what might be expected was in a paper that had recently been published by Carroll (1943) but that paper dealt with pollen distribution in the coniferous forests of the higher Appalachians.

It was obvious that this was a problem subject to experimental study provided the proper situation could be obtained. Fortunately a fairly satisfactory set of conditions was available during the 1946 pollinating period of *Pinus echinata* Mill. The information obtained at that time and presented here is suggestive of what one may expect in mass dissemination of pine pollen in non-mountainous country.

The location at which the field work was done is well suited to the study. The topography is the rolling upland of the piedmont plateau near Raleigh, North Carolina. A rather extensive forest in which *Pinus echinata* predominates lies directly west of a large area of open cultivated land devoid of pines. The center of this open area is more than a quarter of a mile from pines in any direction and except on the west there is no continuous pine forest for well over that distance. The margin of the short-leaf pine forest is abrupt and presents a broad, straight front extending both north and south of the site at which the study was made.

METHODS

Slides with a coating of glycerine gelatin were placed on the ground in a horizontal position and left for 24 hours. They were completely exposed with no

roofed shelter over them. The stations at which they were exposed were located along a line extending eastward from the forest margin toward the center of the open farming area. One station was located within the forest directly under the trees. The others were 0.1 mile, 0.2 mile, and 0.25 mile respectively from the margin of the forest. This was as far as it seemed desirable to extend the series from the forest margin in view of scattered groves of trees toward the east. Duplicate slides were placed at the station 0.25 mile from the forest and the results given for that point represent the average of the pollen falling on the two.

The study was continued over a period of four days at the height of the pollinating period of the short-leaf pine. This was from April 12 to 16. The slides were changed at 5 p.m. each day. Since the slides were not protected the

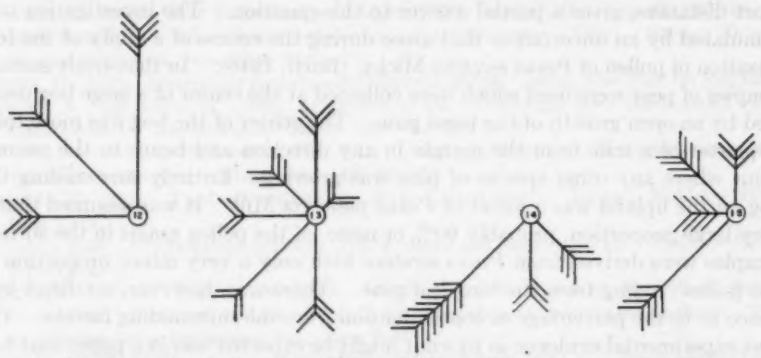

FIG. 1. Wind roses for the four days during which the slides were exposed. The number in the circle refers to the day on which the period starts, the wind rose on the left being for the twenty four hours beginning at 5 p.m., April 12, and so on for the four successive days. For further explanation see text.

study was necessarily discontinued as rain set in during the 24-hour period beginning at 5 p.m., April 16.

The number of pine pollen grains was counted in 25 systematically distributed low-power (100×) fields per slide following the method suggested by Dahl and Ellis (1942). After a count of the pollen grains was obtained from the slides, the figures were then converted to per cent, the amount of the pollen that accumulated on the slides in the forest each day representing 100% for that day.

In order to show the wind direction and velocities for this period wind roses were constructed (fig. 1). These are based on data obtained from the U. S. Weather Bureau Station which is located in the western part of Raleigh about a mile and one half from the site of the field work. They were constructed to show the direction, duration and average velocity of the wind during each 24-hour period that the slides were exposed. The wind rose at the left shows the average wind velocity for the 24 hours beginning at 5 p.m., April 12. In this particular wind rose the longest arrow, which is at the top, indicates that the wind blew longer (10 hrs.) from the north than from any other direction. The

barbs on the arrow indicate the average velocity. In this case it is seven miles per hour, each barb signifying one mile per hour. Similarly the wind blew from the northwest for 8 hours at an average velocity of 6 miles per hour and from the

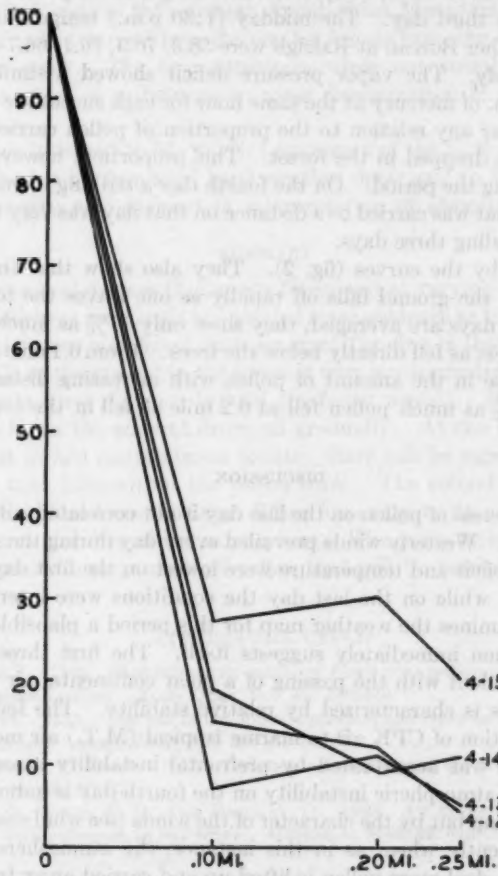

FIG. 2. Relative amounts of pollen collected on slides along a line extending east from a forest margin. The amount of pollen is expressed in terms of per cent of that deposited on the slides placed in the forest. The distances are given in fractions of miles from the forest margin, 0 representing the location within the forest. Each curve is for a twenty-four-hour period beginning at 5 p.m. on the day indicated and continuing until 5 p.m. the following day when the slides were taken up.

west six hours at an average velocity of 6 miles per hour. The other roses represent the wind conditions for the three other successive 24-hour periods.

RESULTS

Although it may be entirely a matter of coincidence with the peak of the pollinating period of the species, there is a noticeable correlation between the

total pollen collected on the slides each day on the one hand, and the temperature and vapor pressure deficit on the other. For the four successive days the total pollen on all slides was 163, 259, *710*, and 510 grains respectively, the peak being reached the third day. The midday (1:30 p.m.) temperatures recorded at the U. S. Weather Bureau at Raleigh were 58.3, 70.3, *76.1*, 66.7 degrees Fahrenheit respectively. The vapor pressure deficit showed a similar curve: 9, 14, *15*, and 10 mm. of mercury at the same hour for each successive day. These factors do not bear any relation to the proportion of pollen carried away from the forest to that dropped in the forest. This proportion, however, does vary significantly during the period. On the fourth day a striking increase occurred. The proportion that was carried to a distance on that day was very much greater than on the preceding three days.

This is shown by the curves (fig. 2). They also show that the amount of pollen settling to the ground falls off rapidly as one leaves the forest. If the data for the four days are averaged, they show only 17% as much pollen at 0.1 mile from the forest as fell directly below the trees. From 0.1 mile out there was a gradual decrease in the amount of pollen with increasing distance from the trees. Thus 15% as much pollen fell at 0.2 mile as fell in the forest and 10% at 0.25 mile.

DISCUSSION

The wider dispersal of pollen on the last day is not correlated with wind direction or humidity. Westerly winds prevailed every day during the study. Both vapor pressure deficit and temperature were lowest on the first day and highest on the third day while on the last day the conditions were intermediate. If, however, one examines the weather map for this period a plausible explanation of the phenomenon immediately suggests itself. The first three days of the study were coincident with the passing of a polar continental air mass (CPK). Such an air mass is characterized by relative stability. The fourth day witnessed the transition of CPK air to marine tropical (M.T.) air moving in from the south. This was accentuated by prefrontal instability associated with a cold front. The atmospheric instability on the fourth day is indicated not only by the weather map but by the character of the winds (see wind rose on the right in fig. 1). Evidently when, as in this instance, the atmosphere is relatively turbulent a great deal more pollen is lifted up and carried away from the forest than when the atmospheric conditions are relatively stable. In this case it amounted to doubling the proportion of pollen carried to a distance of a quarter of a mile from the forest. Obviously the relative amount of pollen moved from the forest may differ appreciably from year to year, depending upon whether the height of the pollinating period coincides with a period of relative atmospheric stability or a period of turbulence. In this connection it is interesting that, in studying air-borne pollen in the Twin Cities area, Minnesota, Rosendahl, Ellis and Dahl (1940) found that the incidence of pollen grains could in many instances be related to severe atmospheric disturbances.

Incidentally the comparison between pollen dissemination in the vicinity of

a cold front and pollen dissemination in a mountainous region is striking. On the piedmont we found that, upon the approach of a cold front, 20% as much pollen fell a quarter of a mile from the forest as fell in the forest itself. Carroll, working in the spruce-fir forests of the southern Appalachian Mountains, found an average of 23% of pollen carried into the conifer forests from the deciduous forests some distance away. The two situations, while not strictly analogous, do emphasize the effect of turbulence on mass dissemination of pollen.

The author is indebted to Mr. C. E. Lamoureux of the U. S. Weather Bureau at Raleigh who kindly furnished local weather data, and to Dr. E. R. Biel of Rutgers University who assisted in interpretation of atmospheric conditions.

SUMMARY

Pollen collected on slides within a pine forest and at varying distances up to a quarter of a mile from its margin has yielded some evidence as to mass dissemination. From this, two significant facts emerge: The first is that the quantity of pollen that falls to the ground in the forest is very much greater than that which is carried even a short distance beyond the forest margin. Beyond one tenth mile from the forest the amount drops off gradually. At one quarter of a mile from the forest in non-mountainous country there can be expected about 10% of the pollen that falls within the forest itself. The second fact is that the amount of pollen lifted from the forest differs from time to time and is dependent upon the relative stability of the atmosphere. The more turbulent the atmosphere the greater the proportion of pollen that will be carried away from the trees.

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THE FORMATION AND STRUCTURE OF THE ZOOSPORES IN ALLOMYCES*

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Plates 22–26

One aspect of the life of *Allomyces* has been but lightly touched: zoosporogenesis. Barrett (1912) watched the process and gave a short account, and McCranie (in Sparrow, 1943) made some sections. Hatch (1935) and Emerson (1941) were interested in phases of its life other than the zoospores. One portion of this investigation has been an effort to bring to completion our knowledge of at least the morphological story of zoosporogenesis. A second phase of the research has been on the structure of the swarmers. As has been postulated repeatedly, relationships between species are commonly brought out by a study of reproductive cells, the structure of spores giving clues to phylogeny of groups, and this is particularly true of the Phycomycetes. The structure of the flagella in a number of forms has been studied by Couch (1941). Though the internal structure of zoospores in the Phycomycetes has been described in several genera, there are not enough morphological data available at present to allow critical comparison of the zoospores in the entire class. The information presented here should contribute to the completion of such a comparison. In the zoospores of *Allomyces*, several inclusions occur in addition to the usual nucleus and nucleolus, which in themselves have some interesting characteristics. The constitution of the lipoid droplets has never been elucidated and the side body is an entity of unknown nature reported in *Allomyces* by Teter (1944) and seen in this genus by Couch (unpublished notes). The most controversial of the inclusions is the nuclear cap, whose origin, composition and function are in doubt. It has been considered as part of the nucleus by Thaxter (1896), Cotner (1930), and Sparrow (1933), and as an extra-nuclear body by Barrett (1912), Kniep (1929), Hatch (1935) and others. It has been described as a food-body by some investigators, as a sex-determinant by Hatch (1935), and as an aid to cilium formation by Debaisieux (1919). Debaisieux (1919) and Hatch (1935) both believed it came from a fusion of mitochondrial elements, but others, notably Karling (1937) and Hillegas (1940), have questioned this explanation and Guilliermond (1941) said, "The mitochondrial origin of this nuclear cap seems still to demand some verification." Since the desirability of more evidence on this problem is clear, it has been one of the aims of this study to learn as much as possible about the

* This paper was condensed from a dissertation presented to the faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the department of botany. The problem was suggested by Dr. John N. Couch and carried out under the direction of Dr. Earl H. Newcomer.

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nature of the nuclear cap in *Allomyces*, that is, to reinvestigate its origin cytologically in living material and with diverse fixatives, to determine its relative specific gravity, its constitution by means of microchemical tests, its optical properties, and its fate, and to compare it with other cytoplasmic inclusions.

MATERIALS AND METHODS

Preliminary tests on fixations were made on *A. javanicus* isolated by the author from soil under cypress trees (*Taxodium distichum*) in Chapel Hill, N. C., but the bulk of the work was done on three species of *Allomyces* furnished by Dr. J. N. Couch: *A. javanicus* Kniep and *A. anomalus* Emerson collected locally, and a culture of *A. arbusculus* Butler which was a descendant of the one used by Hatch (1933, 1935, 1938) and later designated North Carolina 2 by Emerson (1941). All the figures were made from *A. arbusculus*.

The story of zoospore formation and the structure of zoospores were determined as far as possible in live, unstained material. Thalli were mounted in hanging drops over a glass ring about two millimeters high and watched with a Zeiss Apochromat 70× water immersion lens of N.A. 1.25.

Of the many vital stains which have been tested, two are generally reported as least toxic: neutral red and Janus green B. Neutral red was first made up in a 0.005% solution in distilled water, but this caused the death and general staining of the hyphae. Suspecting that impurities in the dye might be causing the evil effects, I prepared a purified lot of neutral red in the following manner, modified from Bensley and Bensley (1938). To 200 ml. of 0.5% aqueous solution of neutral red, add enough 5% KOH to precipitate the dye. Filter and collect the orange colored precipitate. Wash with distilled water and filter again. Evaporate filtrate over a water bath, add about 25 ml. of chloroform to dried residue. Decant the supernatant fluid and discard any dregs. Add enough concentrated HCl to produce an intense color, due to the hydrochloride of the dye, and evaporate to dryness once more. The dried residue is a purified neutral red. This done, the percentage of dye was decreased to 0.002%, then to 0.001%, 0.0002%, 0.0001% and 0.00005%. These solutions were then made up in lots of five each and adjusted to pH 4, pH 5, pH 6, pH 7 and pH 8, and young vigorous cultures were set in each dilution at each pH value. Tests were made in triplicate.

Vacuolar staining occurred only in solutions of pH 7 and in a concentration of 0.0002% neutral red. In other combinations of pH and concentration, the hyphae either took no stain at all or else stained *in toto* and died. Little more could be seen in vitally stained thalli than in unstained ones, though it was interesting to note that nuclear caps concentrated the dye much more successfully than did the vacuoles. In unpublished notes Couch has recorded the same phenomenon.

Janus green B was tried from two sources: Grübler, and Coleman and Bell. Both showed lethal effects at 0.001% (1:100,000), and consequently an attempt was made to purify one lot (Coleman and Bell) according to the method of Bensley and Bensley (1938), who recommend precipitation of the dye with ammonium carbonate to get rid of the zinc which accompanies commercial

Janus green and which is responsible for its toxicity. This method, for still unexplained reasons, failed to produce a water-soluble dye, and a new approach had to be devised. This would be desirable anyway since zinc carbonate is slightly water-soluble in alkaline solution. A satisfactory Janus green can be produced by bubbling H_2S through 200 ml. of 0.5% aqueous solution of the dye to throw out of solution the zinc as white zinc sulfide. To prevent the adsorption of the dye by the precipitate, a few ml. of 95% ethanol can be added to the solution if necessary. The liquid is filtered, and the filtrate, upon drying, leaves purified crystalline Janus green. Concentrations of the dye were made up in lots of 0.001%, 0.0002% and 0.0001%, and adjusted to pH 5, pH 6, and pH 7. A concentration of 0.001% at pH 7 was satisfactory for staining mitochondria, but after several hours in such a solution, vacuoles began to stain, changing as they did so the blue tint of Janus green to violet. The protoplasm would then disintegrate. Nuclear caps take the dye only faintly. The strongest concentration of color appears in the least expected part of the cell: the emergence papillae. These became so deeply stained in the 0.001% solution that with a 16 mm. objective they appeared blue-black against the practically uncolored sporangium.

Two useful stains which might be called lethal stains are gentian violet and methyl green. These, used alone, kill and stain in one operation. Lugol's iodine-potassium-iodide could be classed with them, although it is quite weak as a stain. Gentian violet was used in very low concentration, with only a tinge of color in the solution, but enough to stain the cell contents. Methyl green, commonly used as a chromatin stain on fresh material, was used in rather strong solution. As much of the crystalline dye as could be held on the end of a needle was stirred into a drop of water on a slide. This dark-colored solution was acidified by adding a minute amount of dilute acetic acid from a capillary pipette, and this mixture was then drawn under the cover glass with a piece of filter paper. The dye was prepared thus fresh each time it was used. This produces no visible distortion, but stops all movement of cytoplasm and spores immediately and soon stains the nuclei plainly. Nucleoli in young sporangia and hyphal tips are not stained by methyl green, but those in older portions of the hyphae frequently become quite blue. Nuclear caps stain more strongly than any other cell parts, though ripening sporangia do ordinarily take on a general, diffuse color. The cytoplasm of the hyphae and of young sporangia is stained a diffuse blue, but as the sporangia approach maturity, they stain with a decidedly violet hue which contrasts strongly with the blue of the hyphae. Lugol's solution was as good as gentian violet for bringing out flagella, and was useful in making visible the side body in the zoospores.

Since a satisfactory polarizing microscope was not available, one was improvised. For a polarizer, three disks were cut, one from a sheet of film "Polaroid", one from a lantern slide plate, and one from a 50 x 75 mm. cover glass. The three disks were cemented together with Clarite and inserted on top of the substage condenser of the microscope. The analyzer was made by cementing a smaller disk of "Polaroid" between 17 mm. circular cover slips and placing it above the field lens in the ocular. Under oil immersion, extinction of light

was not complete, but with lower powers the result was quite satisfactory for the determination of anisotropic materials, at least as good as the standard modern petrographic microscope which was used for comparison.

Thalli in all stages of development were centrifuged at 5200 times gravity. After an hour, the rotor was stopped as quickly as possible without jarring, and the contents of the centrifuge tubes were fixed immediately with 2% osmic acid to prevent a reorganization of the protoplasmic parts, since in some stages the zoospores behave very much as Amebae do, and the latter are known to have the ability to readjust themselves within a few seconds after being centrifuged. Layering was then observable in all parts of the thallus.

A relatively new type of microscopy is that known as phase difference or phase contrast or simply phase microscopy, as described by Bennett et al. (1946). In a study involving such small transparent objects as mitochondria, an increase in contrast of the microscopic image is highly desirable. Through the courtesy of the Bausch and Lomb Optical Co., thalli of *Allomyces* were observed with one of the pre-production models, and a photograph obtained of the hyphae. With bright-field microscopy the mitochondria appear as shining particles, but surrounded with a bright halo of refracted light. With the phase microscope, and the phase plate which was available, the mitochondria appeared blue-black as though stained. Fig. 28 is a photomicrograph of a live, unstained hypha of *Allomyces* taken with the experimental equipment and is interesting not only in its connection with the immediate problem, but because it is one of the first photographs of this type taken for purely biological reasons by non-military American workers.

Prolific production of zoosporangia had to be assured. In order to obtain many active zoosporangia simultaneously, several methods were tried, but the one which made the greatest number of sporangia in the least space and in the shortest time was to grow thalli on small pieces of hemp seed cotyledons, about one millimeter in diameter, to allow these to grow vegetatively for several days at a room temperature of about 20°C., then to put them in fresh distilled water and raise the temperature to 27°C. Growing the cultures continuously at the higher temperature produced plentiful sporangia, but they matured a few at a time over a considerable period. For watching or for killing, it is convenient to have many sporangia developing at once.

The fixing fluids tried were representative of several basically different kinds, some fixers being used because they had been used by previous investigators on similar problems, and a repetition of results was sought. The most satisfactory mixture of the Nawaschin type was a weak CRAF solution at pH 1.6.

Solution 1.	chromic acid	0.5 grams
	glacial acetic acid	3.5 ml.
	water	96.0 ml.
Solution 2.	neutral formaldehyde	15.0 ml.
	water	85.0 ml.

For some special purposes osmic acid is useful, as for instance in immobilizing zoospores for study in polarized light or for quick fixation after centrifuging.

Lugol's iodine-potassium-iodide at pH 3.6 serves a similar purpose, and has the added advantage of imparting a light stain, thereby making visible pale parts such as the flagellum and the side body. A small portion of the fluid was added by means of a capillary pipette to the slide bearing the material, and examined immediately. Mitochondrial fixatives used were Meve's, Regaud's, the Zirkle-Erliki mixture (Zirkle, 1934) and Benda's. Others, for comparison, were salted formalin (deRezende-Pinto, 1947), and Meyer's modification of Schaudinn's fluid (Lee, 1928). Since the nuclear cap reacts like the nucleolus to stains, some sporangia were killed in the following solution which at pH 1.8 fixes nucleoli in such a manner as to permit strong staining with Heidenhain's haematoxylin:

CrO_3	2 gm.
propionic acid	1 ml.
$Ni(OH)_2$	excess
water	200 ml.

A killing solution which in higher plants fixes the cytoplasm *in situ* and so preserves the shapes of vacuoles is

$Cr_2(SO_4)_3 \cdot 15\ H_2O$	5 gm.
40% formaldehyde	10 ml.
$Cu(OH)_2$	trace
water	90 ml.

This fixer was employed on *Allomyces* because of the importance of vacuoles in delimiting spores and because the nuclear caps partake in some ways of the character of vacuoles. It was thought that additional information on the nuclear caps might thus be obtained.

The sectioned material was stained in Heidenhain's iron alum haematoxylin or in Flemming's triple stain, using 12 hours in freshly prepared safranine 0 and an hour in crystal violet. The Feulgen technique was carried out after fixation with both a Nawaschin type and a Schaudinn type fixative. The stain was prepared and sections run according to the directions of de Thomasi (1936), Scanlan and Melin (1937), and Coleman (1938).

THE SPORANGIUM-BEARING HYPHAE

Zoosporangia are produced singly or in chains at the ends of hyphae. The ends of the hyphae, all potential sporangia, are consequently objects of interest in a study of zoosporogenesis. Fig. 1 shows the inclusions visible in a live, untreated hypha.

The most conspicuous particles are the multitudinous microsomes which have been called fat bodies, lipoid granules, lipoid droplets, etc. Physically, they are in the form of tiny spheres, and they are liquid, as can be seen by their confluence when they are treated with anything that reduces their surface tension. Chemically, they contain some true fat, but they are not homogeneous.

There is incomplete agreement among authorities as to terminology but the term "lipoid" is used so loosely and so generally that its application here, being

conservative, is safe. Many biochemists refer to any fat-like material as lipoidal, but the word, literally meaning fat-like, has been sometimes used to distinguish between those compounds which merely resemble fats and the true fats which are the esters of glycerol and fatty acids. Currently, however, a substance may be called lipoidal if it is a true fat as an oil or wax, or if it is lecithin or a phospholipid, or a sterol.

In the fungi, for example in yeasts, true fats are known to exist, but the inclusions variously called fat globules or lipoid granules are generally so called because they appear as refringent droplets, and not because an effort has been made to identify them. Sudan III, which stains all substances of a fatty nature, was used as a preliminary presumptive test, and the droplets throughout the thallus stained deep orange-yellow, whether they were in the old hyphae, in the new hyphal tips, or in completed zoospores. Nile blue sulfate stained the bodies a definite blue in all cases, with no hint of pink or violet, thus eliminating the possible presence of fatty acids or mixtures of fats and fatty acids. Fatty acids do not apparently remain in any appreciable quantities in the cells during the synthesis of the droplets. The isotropic appearance of the droplets in polarized light indicates the presence of true fats and the absence of fat-like substances as for example phospho-lipids. When the droplets are finely dispersed, they can be seen between crossed Nicols, but this is probably due to diffraction of light n the surfaces of the tiny particles as occurs in atmospheric haze rather than to an anisotropic condition, for when many of the droplets coalesce to form a large globule 10 microns or more in diameter, the material is definitely isotropic. Sudan IV (Scarlach R) stains the droplets slowly but definitely red, and with the addition of H_2SO_4, the red droplets fuse into the amorphous blue masses characteristic of fats when so treated. In osmic acid, the droplets blacken heavily after about twenty-four hours. When saponified with equal parts of 20% NH_4OH and saturated aqueous KOH, the fat produces masses of sphaerocrystals but no needles (Fig. 44).

All the above-mentioned tests indicate the presence of true fats, and the tests are sufficiently varied and inclusive to show that the bulk of the lipoid droplets is fat. But there is something more. Simple fats are soluble in ether and acetone, yet after 12 hours in ether, the hyphae contain quantities of material which is still stainable in Sudan III, and cold acetone after a similar period reduces the size of the lipoid droplets without removing them. Alcohol has the same effect. Treatment with ether, or with a mixture of alcohol and chloroform dissolves the droplets completely in 12 hours. These mixed results indicate that the lipoid droplets, though essentially fatty in nature, possess in addition a substance, a matrix or surface membrane, of a different nature which resists the solvent action of ether and acetone. The droplets, like true fats, are insoluble in glacial acetic acid. Karling (1937), having made some tests on the microsomes of *Cladochytrium*, formed no positive conclusion about the fatty portion, but decided that they too contain some non-fatty compounds.

After the lipoid droplets, the next most conspicuous inclusions are the nuclei, whose outlines are distinct in untreated material. They are numerous through-

out the hyphae, but especially in the tips. Without stain, a nucleus appears as a clear sphere, containing a nucleolus, but otherwise homogeneous. It is slightly more refractive than the cytoplasm, and because of this and its convex surface, it can function as a microscopic lens, magnifying the mitochondria which pass below it. Methyl green brings out a small amount of chromatin, such a minute quantity that it is visible only in optical section where it is seen through a relatively greater depth than on the upper and lower surfaces. No reticulum is apparent with manipulation of the fine focus adjustment, although the Feulgen reaction does bring out a thin, diffuse, general surface covering in the nuclei. With Flemming's triple stain, an extraordinarily fine chromatin network can be seen in violet over the red nucleolus. In hyphal tips, some nuclei are in the resting state, and a few are dividing, as has been shown by Barrett (1912) and Hatch (1935). The bulk of the nucleus shows no peculiarities, but the nucleolus is a heterogeneous body, variable in shape and content. Nucleoli are generally but not always spherical, and they contain portions which appear sometimes lighter and sometimes darker than the background material. The nucleolus may have several vacuole-like inclusions, or light-absorbing granules, or it may have two well-defined regions, as in Figs. 18 and 64. It is the posterior part to which in the zoospore the flagellum is attached, yet in the hyphae, even before the sporangia are formed, the two-parted nucleolus may be present. Both portions are apparently acid in nature, as they both take basic stains, but they are not identical, since the anterior part stains with crystal violet while the posterior part stains red with safranin. In this same inexplicable fashion the metaphase chromosomes can be stained with violet while the resting chromatin and nucleoli generally stain red. With haematoxylin the heterogeneity of the nucleolus generally is invisible. When fixed in chrome-propionic-nickel, which is known to cause the nucleolus in higher plants to stain heavily with haematoxylin, the anterior portion of the nucleolus in zoospores swells and becomes deeply stained while the posterior portion fails to stain at all. Since this fixation yields large dark nucleoli in the hyphae, it is probable that the posterior portion in the zoospore, being unstained, is not truly nucleolar in composition.

In old hyphae, vacuoles are large and conspicuous, but in the growing ends of young, healthy hyphae, the vacuoles are small and scattered. Only if they are moribund or existing in unfavorable conditions, as for example in a temperature below the optimum, do hyphal tips exhibit distinct vacuoles. There are some vacuoles throughout the thallus at all times, however. In the maturing sporangium they become conspicuous, first as scattered particles and then as anastomosing channels which separate the zoospore initials from one another. I have never seen vacuoles in the cytoplasm of normal zoospores, but when they are placed in hypotonic media, something in the cytoplasm swells, and may even break out of the spore and float away. Though I have not seen them in normal spores, I have interpreted these cell parts as vacuoles, and assume that very small vacuoles do exist in the normal spore. Neutral red shows nothing in the cytoplasm of the zoospore, but does accumulate in the nuclear cap. This probably has little significance beyond the fact that the nuclear cap has such an

affinity for basic dyes that it takes up neutral red with the same avidity with which it takes up methyl green, safranine, etc.

The mitochondria in *Allomyces* are of interest not only because they have been described as forming the nuclear caps in the zoospores, but also because they are unusually large and easy to observe in untreated hyphae. In a live hypha they are long, slender inclusions, sometimes reaching a length of 150 to 200 microns, but not exceeding a fraction of a micron in thickness. They move, twist, turn, fragment, and cause the lipoid droplets, which line up alongside, to have the appearance of strings of beads (Figs. 1, 2 and 28). They are most conspicuous in those portions of the thallus which are not too well provided with lipoid droplets, as in the basal parts and in the very ends of the hyphae, and they are commonly visible in the surface layers of the cytoplasm where they make an ever-changing layer of filamentous particles. In dark field, they are shining white; in polarized light they are invisible. Under correct conditions of concentration and pH, the mitochondria stain with Janus green B. When so stained, they look as they did before staining except for the color. They are in the same places and in no others, and they have the same form. The only advantage gained in staining them is the slight increase in contrast between the blue of the stained inclusions and the colorless or grayish yellow of the rest of the cytoplasm. This can be further intensified by a yellow filter such as a Wratten G No. 15, but even with optimum visibility and distinct staining in the hyphae, I could never see any mitochondria in those sporangia which had developed to the stage when rings of lipoid droplets are visible. No sporangia ever developed from a rudiment to dehiscence in a Janus green solution, and no coalescence of vitally stained particles was ever observed. Hillegas (1940) had the same experience with *Endochytrium*.

In fixed and stained material the mitochondria maintain their form. Fig. 1, drawn from untreated material, Fig. 33, photographed from a hypha killed in a mitochondrial fixative, Fig. 28, photographed from untreated material with the phase microscope, and Fig. 31, photographed from a hypha killed in a Nawaschin-type fixative, all show essentially the same long, filamentous inclusions. Similar results, in which mitochondria were preserved by fluids much more acid than the usual mitochondrial fixatives have been reported for *Saprolegnia* by Guilliermond (1920) and for *Pythium* by Saksena (1936). That the mitochondria of *Allomyces* are chemically rather than physically resistant is shown by Fig. 30, a photograph of a portion of a hypha which had been centrifuged. Nuclei have been forced to the heavy side of the hypha and fat droplets to the light side, with undifferentiated cytoplasm between. There is no indication of mitochondria. The delicate nature of mitochondria is well known and can be readily demonstrated by pressing the cover glass on an aqueous mount.

In studying the optical properties of the lipoid droplets, I noticed anisotropism in the *Allomyces* walls. Since chitin is supposed to be isotropic, I wondered if there were perhaps demonstrable materials other than chitin in the wall. Chitin was reported as a cell-wall substance in *Allomyces Kniepii* by Nabel (1939), the decision being based on the I_2KI-H_2SO_4 test. To make certain of the

chitinous nature of the wall, I made a chitosan preparation by extraction of thalli in 50% KOH at 160°C. over a glycerine bath for 30 minutes. After this treatment, I_2KI with H_2SO_4 gives a deep reddish purple indicative of chitin. Scattered cells from the cotyledons of the hemp seeds, which served as substrata for the thalli, showed a strong blue color. Chitosan can be dissolved in 3% acetic acid, and then precipitated as a nitrate or sulfate with a suitable acid. Fig. 41 shows a sphaero-crystal of chitosan sulfate, produced by the action of H_2SO_4 on the dissolved chitosan, as seen between crossed Nicols. There is, then, positively chitin in the wall of *Allomyces*, but after the chitosan treatment, the walls were still clearly anisotropic. This led to the supposition that some other materials were present, possibly callose, cutin, suberin or cellulose, all of which are also anisotropic. Callose is soluble in 2% KOH. Since this reagent apparently dissolved nothing, the possibility of the presence of callose was ruled out. Both cutin and suberin swell, become deformed, and exude droplets when treated with Schulze's reagent. *Allomyces* walls showed no such reaction. These substances, further, are stainable in Sudan III and Scarlach R, but the *Allomyces* wall is unaffected by these dyes. There is little likelihood of the presence of cutin or suberin. It was a possibility that the anisotropic substance was being masked by the presence of pectic material, as Thomas (1942) found in *Pythium*, yet the use of Ruthenium red as a presumptive test for this substance was negative except in the walls of the resistant sporangia where it was strongly positive. The walls of the vegetative hyphae and zoosporangia seem to contain no pectic material, but the walls of the resistant sporangia do. The test for cellulose with iodine-sulfuric-acid was still negative after the initial KOH treatment, and the mycelia were replaced in the KOH solution and kept there for 100 hours at 60°C. in an effort to dissolve out any fatty masking substance that might be present. The walls were still anisotropic and the cellulose test negative. The possibility of a fatty acid mask over the cellulose was becoming fainter. The mycelium was subsequently treated for a week in ammoniacal cupric hydrate. The reagent dissolved lens paper almost instantly, and filter paper or cotton cloth in a few minutes, yet had no visible effect on the walls of *Allomyces*, which came out of the solution after a week as clear and as strongly anisotropic as ever (Fig. 39). Even after an additional month in 50% KOH the I_2KI-H_2SO_4 test showed a definite chitin reaction. A comparison with cell walls of known cellulose composition can be made in Fig. 40, which shows hyphae, oogonia and oospheres of *Achlya* photographed between crossed Nicols. All efforts to demonstrate cellulose having failed, it became clear that either some masking substance was uncommonly insoluble or else the usual statement of the isotropic property of chitin is not true. Fig. 48 is a photomicrograph of the joint between the femur and the tibia of an insect's leg. The two parts were bent so that they formed an angle of about 135° with the joint at the apex. Thus, when the femur was visible between crossed Nicols, the tibia, being in the extinction position, was invisible. Chitin is thus seen to be anisotropic, but it is not as strongly so as cellulose, as is shown by the fact that Fig. 48 required a longer exposure on the photographic plate than did Fig. 40. It is possible that some cellulose, still masked, remains in

the hyphae of *Allomyces*, but since chitin was positively demonstrated, and since chitin is at times anisotropic, such an assumption is at least unnecessary.

MATURATION OF ZOOSPORANGIA

Apparently every hyphal tip on a sporophyte plant will, if it develops normally, become either a zoosporangium or a resting sporangium. As growth of a hyphal tip proceeds, lipoid droplets of various sizes move out toward the tip but do not fill the last few, about 50, microns, but even the extreme terminus contains mitochondria and nuclei. This does not mean that these inclusions are confined to that region, but that the lipoid droplets are so numerous elsewhere that mitochondria and nuclei are seen most easily in the tip. Wherever by chance a space clear of lipoid droplets occurs, mitochondria can be found, and nuclei can most frequently be spotted by their displacement of lipoid droplets.

The first definite indication of an incipient zoosporangium is a swelling of the hypha (Fig. 2). Then the cytoplasmic content of the region increases rapidly, with an influx of lipoid droplets which practically obscure everything else. Except in the tip, nuclei appear only as unbordered clear areas, commonly homogeneous but sometimes showing a darker nucleolus. In the tip, mitochondria and nuclei with limiting membranes and nucleoli are apparent, but the region where such inclusions are visible becomes ever more restricted until within about half an hour the potential sporangium is almost completely filled with lipoid droplets (Fig. 3).

Long before the sporangium reaches its full size, a cross-wall comes in. At the time when the septum is about to be formed, the protoplasm in the base of the sporangium flows about in such a manner as to make a finely granular-appearing double layer (Fig. 21). The new wall is laid down centripetally in the clear region between the layers by irregular deposition of cell wall material. If the growing septum is watched constantly, it can be seen to increase in size by rapid, intermittent spurts, additions appearing in fractions of a second, or between turns of the fine adjustment screw. The accretions are laid down in spots, now on one side, now on another, so that what might be called the left side of the septum may be more advanced than the right side. Protoplasmic particles can be seen passing through gaps in the septum after most, even apparently the central portion, of the septum is complete. At a time when a median optical section shows nothing (Fig. 23), a surface view may show a continuous line across the hypha (Fig. 22), and a view between surface and median optical section shows a broken line (Fig. 24). Even before the septum is complete, some hindrance to the passage of cytoplasmic inclusions is present, for lipoid droplets pile up below the incipient septum. When the wall has finally grown all the way across the hypha, it is thinner in the central than in the peripheral part. About 10 minutes pass in the formation of the septum from the earliest indication to the first complete closure, and about 30 to 60 minutes more pass before the septum becomes uniformly thick, and as heavy as the old hyphal wall. When the septum is new, it bulges toward the base of the hypha, but soon after completion, it is pushed quickly, in several seconds, to bulge toward the sporangium, and it generally

remains in that position throughout the rest of the life of the plant. Between crossed Nicols with a Gypsum Red I filter, submicroscopic particles appear blue when they are parallel to the vibrating plane of the plate, yellow when perpendicular. The position of the colored areas of the septum indicates the orientation of the micelles, and it becomes apparent that the micelles of the septum are laid down with their long axes in a tangential direction with relation to the septum.

For about an hour after the septum is formed, no apparent changes occur. During this time no vacuoles are visible, and nuclei and mitochondria are seen only intermittently. Occasionally a nucleus comes close enough to the surface to show a nucleolus, but there is lack of visible detail in a sporangium at this time. This period ends when a large central clear area comes into being. It is superficially vacuolar in appearance, but it does not have sharply marked borders and I have not been able to stain it with neutral red. In the living condition, mitochondria at this stage can not be seen, but sections fixed in Regaud's and the Zirkle-Erliki fixative show that they are present. Centrifuging at this time throws the lipoid granules to the light side, and makes a layer of granular stuff of unidentified composition between the heaviest and the third heaviest layer of cytoplasm. No nuclei were thrown down, and no mitochondria could be recognized (Fig. 45). It is at this time that the first slight bulge in the wall of the sporangium indicates the position of an exit papilla.

A short-lived spurt of activity ensues. The central area, which I shall for the sake of convenience call a vacuole, produces sinus-like outgrowths which seem to be on the verge of cutting out spores. Vague, cloudy regions appear, as though nuclei were coming to the surface, but well-defined spore initials are not cut out. The indefinite, unstable spore origins are not in actuality even as plain as Fig. 6 shows but I can not with pen and ink, nor even with a photographic print, give a true picture of the shifting cytoplasm, the indistinct outlines, the obscure movements within the sporangium. The first hint of spore origins soon disappears. The central vacuole takes once more the place of the numerous pale areas, the cytoplasm again becomes denser around the periphery of the sporangium, and the temporary organization is lost. New emergence papillae begin to appear quickly, budding out like blisters. In about twenty minutes from the first appearance of the large vacuole, the sporangium has returned to almost the same state it was in before, except that, in addition to growing the papillae, it has undergone some changes in size, first gaining and then losing in volume.

After the first indication of spore origins, the sporangium goes into a quiescent period which may last from half an hour to several days or even weeks. If the temperature is right and the circumnatant fluid fresh, the sporangium may develop directly, but if the air is too cool—it is seldom too hot for *Allomyces*—or if the water is stale, the sporangium may remain in an arrested state either until a change of conditions stimulates it to new activity or until it dies. During this time, the most conspicuous feature of the zoosporangium is the distribution of nuclei with their rings of lipoid droplets. The nuclei arrange themselves close to the wall, sometimes pressed against it (Fig. 38), in such a position as to be easily

seen (Fig. 9). Nucleoli are generally not visible but can be made out at times in their usual position in the nucleus (Fig. 47). The lipoid droplets become paler, so that the whole sporangium takes on a lighter hue. Around each nucleus is a ring of the lipoid droplets, and it is the ring which makes the nucleus visible. Those nuclei which are pressed against the plasma membrane force the droplets out of the contact area, and at least on the side toward the observer, the droplets give the effect of a true ring (Fig. 51), but if one focuses deep within the sporangium, he can see that the droplets cover the nuclei with a three-dimensional coat. This covering is not simply lying about the nucleus because of displacement of the droplets by the nucleus. The droplets are stuck to the nucleus with some definite attachment, as can be seen in Fig. 47. In this sporangium, the free lipoid droplets were forced to the light side centripetally when they were centrifuged, and nuclei, being heavier, were thrown down. But those lipoid droplets which were associated with nuclei were carried down toward the heavy side in spite of their lower specific gravity. At this stage as in other stages, centrifuged sporangia show no mitochondria. This is not surprising since no mitochondria can be found anyway during the time of disappearance of the spore initials, whether one looks at untreated, vitally stained, centrifuged, or variously fixed material. After the sporangium reaches mature size, but before the nuclear caps form, the mitochondria disappear, and no sign of them can be found in that generation from then on. Janus green reveals nothing in a well-developed zoosporangium, though the hyphae all about it may be showing distinctly stained filamentous mitochondria; and sporangia fixed in the most dependable of the mitochondrial killing agents are devoid of mitochondria when killed during the disappearance stage. In view of the ease with which mitochondria are fixed in the hyphae even by surprisingly acid fluids, it seems that if there were any mitochondria in the sporangia they should be preserved.

Vacuoles are variable during the period of disappearance. In live material, nothing like a distinct vacuole can be discerned until the spores are finally about ready to be formed, at which time there seems to be one large central region without lipoid droplets. Fig. 38 is from a sporangium which was fixed in deRezende-Pinto's salted formalin. This sporangium is about in the same stage as the one in Fig. 9 and a little older than the one in Fig. 35, which shows several smaller vacuoles.

During this time of disappearance, a physiological change is taking place which manifests itself in a changed reaction to staining. Vegetative hyphae and young sporangia have cytoplasm which stains faintly blue in methyl green. But after the sporangia develop almost to maturity, they stain purple, and a low-power view of an entire plant shows readily which sporangia are past a critical point which absorbs more of the violet than the blue from the so-called methyl green, which is really a mixture of methyl green and methyl violet.

Once past the stage of disappearance, with its circles of lipoid droplets, the sporangium develops rapidly and the remaining events take normally only a few minutes. After a waiting period lasting from half an hour to several days, the disappearance stage comes to an abrupt end with massing of lipoid droplets into

an indefinite network, with nuclei and plainly visible nucleoli in the interstices. The transition from the state shown in Fig. 9 to that shown in Fig. 10 takes place so quickly that even rough sketches of passing views are impossible. One moment the sporangium is quiet, with thinly scattered lipoid droplets, and droplet-encircled nuclei. The next moment the droplets have been released from about the nuclei and have collected into a definite pattern in anticipation of the final cutting out of spores, and the whole sporangium has decreased visibly in volume. At first there is no separation into discrete spores, the masses of lipoid droplets merely forming boundaries between the spaces containing the nuclei. Within a few seconds, the droplets begin to separate into masses, with clear lines of cleavage cutting the spore initials into polygonal blocks, but there are not, for the time, any distinct lines of demarcation other than the edge of the lipoid droplet region. In other words, when the cleavage first appears, one can not see a real line of ectoplasm which can be represented by a pen-and-ink drawing. There is a difference in refractivity between the materials which will make up the ectoplasm and the endoplasm of the spore. The edges of spore initials are visible before completion because of lines between them which look like the lines in convection currents, as when two miscible liquids of different refractive indices e.g., water and glycerin, are slowly stirred.

While cleavage planes are separating the protoplasmic blocks, the clear region about each nucleus is increasing as the droplets are being pushed back, the nucleus is being forced to one side of the cleared area, and simultaneously the nucleolus is moving to one side of the nucleus (Fig. 11). When the nucleoli first become visible again after their long absence during the disappearance stage, most of them are centric, but during the few minutes required for completion of a spore, the nucleus moves to one side of the spore, and the nucleolus moves to one side of the nucleus. The flagellum, being attached to the nucleolus, is probably formed during this time, but I have never been able to see a flagellum before the escape of the first spore from the sporangium. Hillegas (1940) saw pre-emergence flagella in *Endochytrium* after crushing the sporangia, but premature rupture of the sporangium of *Allomyces*, even if it is done delicately just before the spores are completely delimited, merely forces out through the exit papilla an amorphous mass of cytoplasm in which the only recognizable parts are the lipoid droplets.

The final completion of the spores involves the formation of a distinct limiting membrane and the finishing of the nuclear cap. A few seconds after the first cleavage furrows come in, the spore initials form an ectoplasmic membrane which can be seen momentarily here and there as sharply as a drawn line, and simultaneously the clear area about the nucleus becomes bounded by a membrane which makes the nuclear cap a physical unit. It would be a temptation to think that the nuclear cap had been completed deep in the sporangium and had merely risen to the surface, so quickly does it come into view, but the nuclei can be seen so well for a short while before the caps that such a move could not be. Furthermore, fixed material shows coalescence of particles close to the periphery of the

sporangium. The process is simply a fast one, and the speed is a normal thing. Generally, if more than about two or three minutes elapse between the first appearance of the nuclei as in Fig. 10 and the definite formation of nuclear caps and ectoplasmic boundaries as in Fig. 11, the development is abnormal and the spores will not escape intact.

Typical examples of the time required for delimitation of zoospores are these two which are representative of about a dozen such timed observations.

Observation No. 1:

4:24—spore initials clearly delimited, intermittently visible nuclei with already eccentric nucleoli.

4:25½—clear areas about nuclei.

4:27½—nuclear caps complete.

4:30—first zoospore escapes.

Observation No. 2:

2:15—scattered lipoid droplets, some in circlets, denser at apex of sporangium.

2:28—neat circles of lipoid droplets, central vacuole vague.

3:00—spore origins faint, nuclei and nucleoli visible, but no nuclear caps.

3:02—no change: still no caps.

3:02½—nuclear caps sharply delimited but small.

3:03—nuclear cap mature.

3:07—first zoospore escapes.

Cleavage of the zoospores as seen in fixed material is similar to that described by many previous writers. Karling (1937) and other have demonstrated in a variety of organisms that when spores are cut out, the separating fissures proceed from the central to the peripheral regions. In *Allomyces* cleavage takes place just before the nuclear caps are formed, the furrows becoming visible in live material some seconds before caps can be seen. In fixed material the separation of the protoplasmic portions is complete when the particles of nuclear cap material are still in the form of scattered granules (Fig. 52).

When dehiscence occurs, one of the exit papillae opens. The papilla is a soft, elastic bulge whose apex is made thin and weak, presumably by the local action of enzymes. The bulge, a liquid-filled blister which has a strong and surprising affinity for Janus green, has a cytoplasmic membrane in addition to the sporangium wall. When the exit is about to open, the papilla swells, the apical region of the wall dissolves and, about the edges of the hole, the wall material rolls back into a slightly ragged edge. The exit hole eventually becomes smooth and round. After the rupture of the first exit papilla of a sporangium, the others, of which there may be as many as half a dozen, subside temporarily (Fig. 12), and the zoospores start escaping from the open one. But the others do open soon, showing that internal pressure, which must cease to exist as soon as one opening is made, is not the sole reason for the breaking out of the spores, if indeed it functions at all. Sometimes a spore will start out one papilla before any exit has opened, then draw back and remain in the sporangium until some other exit has opened. Once dehiscence has got under way, the zoospores squeeze out rapidly,

forcing their way through the hole which is ordinarily smaller than the diameter of the spore. The first few escapees remain clumped in a mass of three to a dozen, milling and sliding about but sticking close together. This clumping resembles the exit habit in *Pythium*, but in *Allomyces* I have been unable to discern a distinct vesicle, and since at times a flagellum can be seen waving outside the spore mass, it seems unlikely that any vesicle actually covers the mass.

Wolf (1944) in his generic characterization of *Allomyces* describes "zoospores emerging singly, without formation of a vesicle." Though this is literally correct, it gives the impression that each spore swims away as soon as it comes out, when actually the first few zoospores do remain massed, and look as though they were enclosed in a vesicle. In fact, in view of the great elasticity of the cytoplasmic membrane inside the sporangium wall, it is not impossible that sometimes a true vesicle does enclose some zoospores. The later emergents come out singly and usually swim away directly.

As a parenthetic observation, the dehiscence of the resistant sporangia along preformed lines, as was pointed out by Couch (1945), has been observed, and a photomicrograph (Fig. 50) is included here. It can be seen that a straight row of pits parallels the fissure and indicates the direction and position of the line of dehiscence.

After the first outward rush, the exodus becomes less rapid, some spores requiring a minute or more to make their way out of the opening, frequently being held back by having the free end of the flagellum entangled in the remaining press of spores. How a spore in such a position can maintain tension on the flagellum as in Fig. 13, when the most active part of the flagellum is being held immobile, is an unanswered question. The last spores may remain in the sporangium for half an hour or more, or in many instances may fail to find the exit, in which case they may encyst within the sporangium, germinate, and put out rhizoids through the exit openings. A normal sporangium empties completely in ten to fifteen minutes, but it is not uncommon to see remnants of cytoplasm, incomplete zoospores, left after all the active particles have been evacuated.

It is to be noted that the sporangium does not develop simply by swelling until the spores are liberated and then undergoing deflation. Instead, it increases steadily in size during its early development and reaches its maximum volume at the time of the first appearance of the spore initials. Then there is a decrease which continues until the disappearance stage is reached, this diminution amounting to about 13% of the maximum volume. During the long period of disappearance there is little change, but immediately after the reappearance of the spore origins, while the cleavage fissures are at work, there is a second significant decrease of over 9% in volume from the size at the beginning of the disappearance stage. Altogether, from the time of its maximum size until the sporangium is just ready to liberate its first zoospore, the sporangium loses about 22% of its volume in two moves: one immediately before the disappearance act and another during final cleavage. That these are the result of dehydration is readily agreed. A set of measurements in tabular form is given in Table I in which dimension

changes and approximate volumes are arranged in chronological sequence and correlated with development of the sporangium.

STRUCTURE OF ZOOSPORES

The finished zoosporangium commonly contains about 40 to 60 zoospores, but in a vigorous plant, supplied with abundant nourishment, there may be well over a hundred. If a plant establishes itself on a microscopic bit of food, it may grow only one unbranched hypha several hundred microns long, which terminates in a small sporangium producing a dozen or so zoospores. There is no apparent difference between the spores from a large and those from a small sporangium. When in spherical shape, the spores are about 10–12 microns in diameter, but abnormalities of various sorts result in freaks, ranging from 5 to 15 microns, which I have never seen germinate.

TABLE I

Changes in volume of sporangium

TIME P.M.	STATE OF DEVELOPMENT	LENGTH IN MICRONS	THICKNESS IN MICRONS	VOLUME IN CUBIC MICRONS
2:10	growth of sporangium	74	18	12,550
2:25	growth of sporangium	72	21	16,550
3:10	growth of sporangium	73	23	19,750
3:30	first appearance of spore initials	87	27	33,200
3:35	spore initials	85	27	32,700
3:45	spore initials	84	26	29,600
3:52	spore initials	83	27	31,500
4:04	disappearance of spore initials	82	26	28,800
4:25	maturation of sporangium	82	26	28,800
5:00	continued maturation	83	27	31,500
5:40	second appearance of spore initials	87	26	30,600
5:45	first spore escaping	86	24	25,800
5:51	sporangium almost empty	86	24	25,800

The liberated spores are capable of either flagellar or ameboid movement and their form at a given moment is determined by the kind of locomotion employed. When resting, either temporarily or just before encystment, they are spherical (Fig. 62), but when swimming actively they are ovoid or ellipsoid (Fig. 43). Immediately after escape from the zoosporangium, they move in an ameboid fashion for a short time, from a few seconds to several minutes, and they frequently use this kind of movement between periods of the swifter flagellar locomotion. The temporary reversions to ameboid movement may last from a few seconds to an hour or more, and during such times the spores exhibit typical pseudopod formation and incessant change of shape (Fig. 60).

Some details of spore morphology can be learned from the disintegration of the spores. I have watched this process several times, and a typical example, condensed from a stenographic record, is as follows:

Observation commenced at 12:55 A.M., Mar. 18, 1947. Zoospore comes to rest with flagellum extended, whip-lash visible. Mounted in Janus green B solution of unknown but very low concentration (Fig. 14). Whip-lash seems to make tiny loop. Whether it is a loop or a blister is impossible to know (Fig. 15). Vesicle or loop increases in size as flagellum shortens until it becomes about half as large as nucleus, flagellum at this time being about half its original length (Fig. 16). Vesicle has two dark granules on posterior portion, which on focussing up and down I can see are parts of lines encircling the vesicle. The vesicle buds out from itself a smaller vesicle, and it in turn a third and still smaller one, the least one being about the size of a small lipoid droplet (Fig. 17). (The smaller vesicles were either lost or sunk back into larger vesicle during subsequent movements.) The nuclear cap is distinctly outlined, heterogeneous; vacuoles clear and spherical, plainly visible in cytoplasm. With a jerk, flagellum pulls vesicle close up to spore body. Ectoplasm "explodes" leaving nuclear cap, nucleus and nucleolus floating in water. Cytoplasm, with vacuoles and lipoid droplets, now suddenly showing vigorous Brownian movement, begins to disperse. Nucleus and nuclear cap swelling, the cap becoming less regular in outline and showing more vacuolate structure. Nucleolus of two parts: anterior part dark, posterior lighter. Two vacuoles, with tonoplasts intact, sticking to anterior end of nuclear cap. Flagellum seems to come up and over remains of zoospore. Flagellum bumpy, dark, crooked; attachment, whether to vesicle or to nucleolus, at this time uncertain but more likely to former. (In other cases, flagellum is seen definitely attached to posterior part of nucleolus.) Three strands of dark material (chromatin?) over nucleus, too fine to be represented faithfully in inked drawing even with finest available pen. Nucleus now "explodes" violently enough to spin mass of debris through 90°. Nuclear cap and nucleolus still intact; flagellum thrown to one side (Fig. 19). 1:15 A.M. Flagellum moves, one end coils and uncoils, although by now the old spore is nothing more than a disorganized mass whose parts would be unrecognizable to one who had not watched the disintegration. The nuclear cap begins to dissolve, its outlines becoming blurred and indistinct, but the nucleolus and vesicle are still sharply outlined. The flagellum has become ravelled, and the one remaining vacuole, having floated a little distance away, is no longer smoothly spherical. 1:20 A.M. The only parts remaining apparently unchanged are the lipoid droplets and the nucleolus, whose double nature is now more evident than ever. The clear posterior portion is spherical, and its diameter is about one third that of the entire nucleolus. It fits into the nucleolus in the same way that the nucleolus fits into the nucleus and the nucleus fits into the nuclear cap.

The size of zoospore nuclei. Even a casual observation of the development of zoospores shows that when the spores are complete, their nuclei are smaller than those of the hyphae. In other words, there is a reduction in the size of the nuclei when the nuclear cap is being formed, and that this reduction occurs late in the history of sporangium formation can be seen by comparing the nuclei in Fig. 2 (1:55 P.M.), which is practically a vegetative hypha, with Fig. 9 (4:25 P.M.), which is an almost mature sporangium, and Fig. 12 (5:46 P.M.), which contains

completed zoospores, all these figures being drawn to the same scale. Thinking to determine as precisely as possible what the reduction in size amounted to, I measured a number of nuclei in the hyphae, and a number in zoospores and figured the means for both diameters and volumes. Since the mean diameter of the hyphal nuclei is 5.48 microns, and that of the zoospore nuclei is 3.2 microns, the hyphal nuclei are 1.7 times the diameter of the zoospore nuclei. It is significant that out of 60 measurements, the diameter of the smallest hyphal nucleus is still 1.2 times that of the largest zoospore nucleus. For further comparison, the diameter of the largest hyphal nucleus measured is 2.8 times that of the smallest zoospore nucleus. The volumes show more contrast than the diameters. In volume, the hyphal nuclei average more than 3.2 times the zoospore nuclei. The smallest hyphal nucleus measured contained almost 1.5 times the volume of the largest zoospore nucleus; while at the opposite extreme, the largest vegetative nucleus contained more than eight times the volume of the smallest reproductive nucleus. While these differences do not compare with the enormous differences that sometimes obtain between vegetative nuclei and fusion nuclei, as for instance in basidia, they are nevertheless significant.

In *Allomyces*, then, there is a considerable decrease in size of the nuclei when the nuclear caps are deposited. In general, however, nuclei in reproductive cells tend to be larger rather than smaller than vegetative nuclei, or at least so it seems. To get some numerical data on this aspect of the question, nuclei of fungi were measured in different taxonomic groups from published data which all too infrequently include either measurements or scale-drawn figures of nuclei in all the stages of a plant's growth. When possible, non-sexual spores were used, but sexual spores were taken in most cases because they were available in measurable data while such information on non-sexual bodies is scarce.

In Table II are given measurements of nuclei in six species of fungi which do not have nuclear caps. In these organisms, the diameters of the reproductive nuclei are from 1.3 to 2.2 times those of the vegetative nuclei, and in volume the reproductive nuclei are 2.4 to 11.5 times as great as the vegetative ones. The measurements of three species with nuclear caps are summarized in Table III, from which it can be seen that in diameter, the vegetative nuclei are 1.7 to 2.2 times the reproductive ones, and in volumes, the vegetative are 3.2 to 11.1 times the reproductive. The means of Tables II and III are compared in Table IV, which shows how the ratios between vegetative and reproductive nuclei in species with nuclear caps are the inverse of those in species without nuclear caps. The reproductive nuclei in forms without nuclear caps have a mean diameter 3.4 times that in species with caps, and a mean volume 47.5 times that in such species. In other words, the nuclei in zoospores with caps have a diameter less than a third and a volume almost one fiftieth what might be expected on a basis of what is known of other fungi. In all instances except the species studied here, the data are meagre, and the results would certainly change somewhat quantitatively if more numerous measurements were available, but the general agreement and uniformity of the results indicate that they are probably fairly trustworthy.

During the reduction in nuclear size, the nucleolus also undergoes shrinkage,

TABLE II

Species without nuclear caps

PLANT	SAPROLEGNIA		SORDARIA		OPHIOBOLUS	
SOURCE OF DATA	Trow, 1895		Ritchie, 1937		Jones, 1926	
	Diam., microns	Volume, cubic microns	Diam., microns	Volume, cubic microns	Diam., microns	Volume, cubic microns
Vegetative nuclei (mean)	1.4	1.4	1.9	3.5	0.32	0.017
Reproductive nuclei (mean)	2.6	9.2	2.5	8.4	0.72	0.195
Ratio vegetative to reproductive nuclei	1/1.9	1/6.6	1/1.3	1/2.4	1/2.2	1/11.5

PLANT	PHYLLACTIDIA		TUBER		TREMELLA	
SOURCE OF DATA	Harper, 1905		Dangeard, 1894		Dangeard, 1894	
	Diam., units*	Volume, cubic units*	Diam., microns	Volume, cubic microns	Diam., microns	Volume, cubic microns
Vegetative nuclei (mean)	3.7	27.1	1.3	1.0	1.5	1.7
Reproductive nuclei (mean)	8.1	268.4	2.6	9.1	2.2	5.5
Ratio vegetative to reproductive nuclei	1/2.2	1/9.9	1/2.0	1/9.1	1/1.5	1/3.2

* Actual measurements not given.

TABLE III

Species with nuclear caps

PLANT	CLADOCHYTRIUM		ENDOCHYTRIUM		ALLOMYCES	
SOURCE OF DATA	Karling, 1937		Hillegas, 1940		Author's observations	
	Diam., units*	Volume, cubic units*	Diam., microns	Volume, cubic microns	Diam., microns	Volume, cubic microns
Vegetative nuclei (mean)	26.2	9,442	3.1	15.6	5.5	34.6
Reproductive nuclei (mean)	15.4	1,919	1.4	1.4	3.2	10.8
Ratio vegetative to reproductive nuclei	1.7/1	4.9/1	2.2/1	11.1/1	1.7/1	3.2/1

TABLE IV

	RATIOS OF NUCLEAR SIZES	
	Diameters Vegetative nuc./ Reproductive nuc.	Volumes Veg. nuc./Rep. nuc.
Species with nuclear cap	1.86/1	6.4/1
Species without nuclear cap	1/1.85	1/7.1

* Actual magnifications not given.

but the changes in the latter cell part have not been carefully studied. It may be, however, that the nucleolus is more intimately involved in the formation of the nuclear cap than the nucleus itself, particularly since the nuclear cap in staining reactions at least resembles the nucleolus as nearly as any other part of the cell.

The fact that the deposition of a nuclear cap is accompanied by the diminution of the nucleus suggests that there may be nuclear material in the cap. To test the possible truth of this suggestion, tests were made for several of the substances which might reasonably be expected in a cell inclusion which probably has a similarity to nuclei. The results of the tests will be described below.

The lipoid droplets. The lipoid droplets are the only cytoplasmic inclusions that can be followed by direct observation from the vegetative hypha through sporangium formation, into the zoospore, and on into the next generation. Their distribution is similar in all the swimming stages of the organism: zoospores, R. S. zoospores and gametes, but they are less numerous in the R. S. zoospores and male gametes than they are in the other motile cells. The presence of carotin in the male gametes has been pointed out. Individual spores differ in the distribution of the droplets, some having all of them congregated at the anterior end, others having them generally dispersed, but the common distribution is as follows: the greatest aggregation of droplets is in the anterior end where about three fourths of them stay when the spore is swimming, while a second smaller clump collects at the posterior end near the rhizoplast, and a few more are scattered throughout the cytoplasm about the nuclear cap and close to the side body. In a spore in the ameboid stage, the droplets are moved about indiscriminately wherever the streaming currents of cytoplasm carry them.

The side body. An inclusion which has been described in several species of the Blastocladiales, but whose structure and function are not understood, is the side body, sometimes called the Seitenkörper because its first description was in German. This particle has been found in the zoospores and gametes of *Allomyces*, *Blastocladiella*, and *Catenaria*. Debaisieux (1919) saw and illustrated a structure quite similar to the side body in *Coelomycidium*, but he did not describe it (see his Fig. 31), and no such organelle appeared in the literature again until Stüben (1939) published his description of *Sphaerocladia*. It is somewhat sausage-shaped, and occupies the space beside the nuclear cap, extending from the posterior end of the swarmer for a variable distance toward the anterior end. In some of the figures of *Blastocladiella*, Couch and Whiffen (1942) show side bodies in some spores almost as long as the body of the spore. In others, and in *Allomyces*, the side body is shorter, attaining a length of only about half the length of the spore. Teter (1944) found them in the R. S. zoospores of *Allomyces cystogenus*, and I have found them in the zoospores (Fig. 43) and R. S. zoospores of *A. arbusculus*, where they can be seen with lipoid droplets almost surrounding them. The side body makes a bulge in the side of the spore, giving a one-sided outline to an otherwise symmetrically ovoid shape.

No one has claimed to see a side body in a live swarmer. Those reported previously were found in osmic acid preparations, and the ones pictured here

were observed after treatment of the material with Lugol's solution. When one considers the number of planonts that have been watched, their small size, their transparency, and the ease with which other internal parts can be distinguished, it seems strange that no one should ever see a live side body. This might indicate that the organelle is an artifact, but there are arguments against such a supposition. The side body, as seen and interpreted by various eyes and minds, has a convincing constancy of form and size. It has been discovered only in a small, specialized group of fungi whose swarmers share other definite peculiarities, such as the single posterior flagellum, the lipoid droplets, and the nuclear cap. Furthermore, the fact that a cell part is seen only in fixed material is not proof that the part is an artifact. Questions that remain unanswered, however, are these: why does the side body show up in only a small number of the zoospores and gametes that are examined, and why were they not seen by more of the numerous investigators who have been interested in the Blastocladiales for the last half-century?

The flagellum. Little is known about the formation of the flagellum. For two reasons it may be inferred that it is made late in the development of the zoospores. First, the nucleolus, which is held in an eccentric position in the completed spore by an intranuclear extension of the rhizoplast, can be seen in a centric position just before the spores are finished; second, premature rupture of the sporangium fails to bring out any isolated flagella or portions of flagella. When Hillegas (1940) crushed the almost mature sporangia of *Endochytrium* he found flagella with vesiculate tips, and concluded that the vesicle was in the act of producing the flagellum. In view of the fact that degenerating flagella are usually the ones which have terminal vesicles, it seems likely that the rude handling accorded the *Endochytrium* flagella may account for their appearance. In sectioned material, the flagella are not visible until the spores are completed. In view of the fact that the flagella, once formed, can withstand relatively rough treatment, it is probable that if they were formed early, they would be found in sectioned sporangia.

The mature flagellum is not inflexible, but it does have some resistance to bending. If one spore is lying quiet, and another spore swims against its flagellum, instead of bending aside, the struck flagellum will cause the attached spore to swing around. When the spore is swimming free, the flagellum seems to vibrate almost as a straight rod, as Couch (1941) has shown in dark-field photographs. But when a spore is restricted in movement, as when only one remains in a sporangium, it can be watched under high magnifications, and when the flagellum is moving slowly, undulations can be seen passing along it as waves move along a suspended rope, the impulse seeming to start at the base of the flagellum. Under such conditions, there are currents set up in the liquid confined in the sporangium, as is shown by the swirling waste particles, and these currents are of such magnitude that the small whip-lash at the end of the flagellum could scarcely cause them. The ability of a zoospore to tug at an entrapped flagellum has been mentioned. In such a case, the whip-lash would be powerless, yet the spore can maintain tension on the flagellum. At times, under such

conditions, the spore trembles, shaking its 4 micrograms or so from side to side. This would indicate that the wave impulse does originate at the base of the flagellum, because if a free-floating body like a zoospore should impart a vibratory motion to a relatively firm but flexible rod like a flagellum, and if the rod were held firm at its nether end, the body would move from side to side. This is further brought out when a zoospore wraps its flagellum about itself and sends slight waves along it, with the result that the spore turns slowly like the earth on its axis. If the impulses were originating at the end of the flagellum, the spore would move in linear translation instead of revolving even though the flagellum were wrapped around the body. I have also seen zoospores go into reverse, using the flagellum as a tractellum instead of a pulsellum, and this is a time during which the whip-lash may be the sole moving part, as perhaps occurs in those motile organisms which have one anterior flagellum.

Some inferences can be made concerning the minute structure of the flagellum from its disintegration. The fact that a vesicle may be formed by the flagellum indicates that it is more likely a tube than a solid rod. If air is forced under pressure into an elastic tube, there will be formed at the weakest place in the tube a blister similar to the ones seen on zoospore flagella. They are usually but not always terminal. The vesicle is not a loop but is indeed a vesicle, as has been described by Fisher (1894), Hillegas (1940) and others. Berdan (1941) has described loops, but in *Allomyces*, in one instance at least, the image of a ring was caused by a vesicle. This is known because there were two fine lines passing up and over its body on the side toward the observer, and these could be focussed on with the fine focussing adjustment so as to demonstrate their curved path (Fig. 16). Only a three-dimensional body could support such a configuration. Previous decisions concerning the degeneration products of flagella have been made subjectively.

There is some evidence that the flagellum is fibrillar in its fundamental structure. The most convincing view of this was seen when the spore represented in Fig. 14 finally disintegrated. I pointed out the stringy appearance of the flagellum in Fig. 20 to my wife, who agreed that my drawing is a faithful representation of the aspect of the preparation. I have been unable to repeat this, and I do not know what factor or combination of conditions caused the fraying, but it was unmistakably clear. Another indication of fibrillar structure was found when zoospores were treated with nitric acid. This reagent sometimes causes longitudinal splitting of the flagellum. Welch, Roseveare, and Mark (1946) used 70% nitric acid to split rayon fibers into their component fibrillae. The technique is not dependable for flagella, but when it does work it is quite successful, and I have demonstrated the partial lengthwise splitting to Mr. Paul Titman, a graduate student in this department. Without explaining what I was seeking, I asked him to draw a spore which I had treated with nitric acid, and his sketch of the flagellum is reproduced herewith as Fig. 27. These fragmentary bits of information, plus the fact that the flagellum is faintly anisotropic (Fig. 42), lend credence to the assumption not only that the flagellum is a tubular rather than a rod-like structure (an idea not infrequently expressed), but that

it is made up of longitudinal fibrillae. The tinsel flagella described by Fisher (1894), Vlk (1939) and Couch (1941) could be readily explained as natural expressions of such structure. It might be profitable to experiment with the nitric acid technique on a variety of flagellated organisms in an effort to discover whether the locomotor organs of other species can be made to reveal additional detail.

There is no agreement as to the fate of the flagellum. Hatch (1938) says "the cilium is thrown off," and (1944) "when R. S. zoospores encyst, the movement of the cilium stops instantly—the cilium is apparently cast." On the other hand, Barrett (1912) mentions retraction of the cilium, and Sparrow (1943) on page 395 says the zoospores of the Blastocladiales "apparently absorb the flagellum into the body." Every spore that I have watched coming to rest waves its very evident flagellum for a while as it moves about in an ameboid fashion, then becomes spherical and wraps its flagellum around itself. Not infrequently it sends waves along this circumferential flagellum as described earlier in this paper, thereby making itself revolve slowly. After this, the flagellum comes into close contact with the body of the spore, and can sometimes be seen lying over the body of the spore across a clear place. What happens to it then I can not say with certainty, for I have never seen a flagellum after this stage. Never have I seen a flagellum cast from a zoospore of *Allomyces*, and if that were a normal occurrence I think I should see it, for the flagellum is readily observable particularly when it moves.

The nuclear cap. The nuclear cap is an uncommon inclusion occurring only in the gametes and zoospores of some of the lower Phycomycetes. It does not have the same character in all genera, being invisible in live swarmers of the chytrids but clearly visible in most of the Blastocladiales. In *Allomyces*, it is seen easily without any special methods of preparation as an elliptical body which fills the bulk of the swarmer. There is space enough in the anterior end of a spore for a layer of cytoplasm containing some lipoid droplets. At the posterior end, the nucleus fits deep into and is inseparably bound with the nuclear cap. The cap is distinct from the nucleus, however, and the nuclear membrane can be seen where nucleus and cap are contiguous, there being a slight difference in refractivity between the substance of the two parts. The cap is colorless, grayish, or sometimes tinged with green. It frequently seems homogeneous in texture, but may also have various inclusions within itself. There may be a single, spherical, vacuole-like object of considerable size, or there may be several such entities, or there may be such a mass of tiny "vacuoles" that the texture of the cap looks foamy. Although the cap is definitely outlined, it is quite soft and pliable, so that when a zoospore undergoes the stretchings and convolutions of ameboid movement, the nuclear cap is contorted with the rest of the protoplasm, entering into pseudopods, elongating and drawing back as the spore changes shape (Fig. 60).

The formation of the nuclear cap as observed in living material has already been described. In fixed, sectioned and stained sporangia, the deposition of the nuclear cap material is seen to be quite different. During the early stages of sporangium formation, the cytoplasm shows no peculiarities, nor do the

nuclei. As growth proceeds, nuclei are carried into the sporangium along with the necessary amount of protoplasm. While the sporangium is young, the number of nuclei is increased by the occurrence of scattered mitoses. The mitoses are not simultaneous. During this time mitochondria are evident, but during the stage when the spore initials have been replaced by the circlets of lipoid droplets about the nuclei, the mitochondria disappear, and can not be seen either alive or fixed. The peripheral arrangement of the nuclei and lack of mitochondria are shown in Fig. 38. They are never as long and conspicuous even in very young sporangia as they are in vegetative hyphae, and it may be that they fragment into such small particles that they become unrecognizable.

After the final cleavage fissures have delimited the spore initials, conspicuous granules begin to appear. They stain heavily with basic dyes, and retain dyes with greater tenacity than any other cell part. In the earliest stages, they are dispersed throughout the cytoplasm, being no denser near the nuclei than anywhere else, and are individually only a fraction of a micron in diameter (Fig. 52). The little particles flow together, forming larger and larger masses, as in Fig. 53 and 54, but still not any more densely congregated about the nucleus than in the peripheral cytoplasm of the spore initial. Only when the growing particles finally fuse into a single irregular unit is a connection with the nucleus made. The nuclear cap, when first deposited, is likely to be lobed and irregular (Fig. 55), but it never becomes separated from the nucleus until the zoospore germinates. It settles down at last to its final shape, representative examples of which can be seen in Figs. 56, 61 and 63.

All the fixatives employed gave practically the same image as far as the formation of the nuclear cap is concerned. Such different killing fluids as Nawaschin's, Regaud's and deRezende-Pinto's may produce images in nuclei so divergent that one would scarcely believe himself to be dealing with a single species. The aspect of the nuclear caps themselves varies with different fixations after it has been completely formed, as in Figs. 58, 61 and 63, yet while it is in the process of condensing, it seems to be indifferent to the method of fixation. Another problem lies in the fact that there are many examples of condensing caps to be found in sectioned material. Of course I always killed thalli during as active a period of spore production as possible, but when one considers the few short moments required for the observable deposition of nuclear caps, the relatively small number of sporangia which must be in precisely that stage at the time of fixation, and the still fewer which would be cut when a thallus is sectioned at 6 microns, it is to be wondered at when even a few stages of nuclear cap formation are found. Contrary to expectations, however, such stages are not uncommon, and it is easy to obtain a close series from the earliest scattering of granules to the entire nuclear cap. This makes me think that the process is slower than observations on living material would lead one to believe, and that the early stages are simply not visible, the final coalescence of cap material being the only portion of the story which can be watched directly. This is obviously conjecture, but it may hint at an explanation as to why the nuclear caps of other genera can be seen only after fixing and staining. If such an organism as *Cladochytrium*

has not gone as far along the evolutionary road as *Allomyces*, its nuclear cap might be in a state of incomplete development which makes it invisible in a live zoospore, but well enough developed to be fixable and stainable.

Physically the nuclear cap is one of the toughest parts of the zoospore. It shrinks upon drying, and unless hardened by fixation it shrinks upon dehydration by reagents, but upon rehydration, particularly in a basic medium, it swells. After disintegration of the outer cytoplasmic layers the nuclear cap is left intact. It retains its original shape, maintains a sharp outline for a while in water, and does not undergo any essential changes in its apparent internal structure. After a denuded nuclear cap has remained in water for some time, its outline becomes vague and the whole cap seems to be gelatinized. This might be due to considerable imbibition which could cause an appearance of partial dissolution. The rupture of a nucleus, leaving the nuclear cap undamaged, shows that the nuclear cap is outside the nuclear membrane, but since under no circumstances have I seen a nucleus separate from its cap, the connection between the two must be very close.

Except for a few tiny granules of unknown material, the nuclear cap is the heaviest thing in the spore, being even heavier than the nucleus. After an hour in the centrifuge at 5200 times gravity, the zoospore has layers with the lipoid droplets at the light side, followed in order by the undifferentiated cytoplasm, the nucleus, the nucleolus at its heavy side, the nuclear cap and finally the unidentified grains (Fig. 62). The flagellum is generally lost by the time layering has occurred, but in one instance, when the flagellum had been retained, it was connected at the side of the spore, considering the light or fat-collecting region as the "top". The internal arrangement of this spore was the same as that in Fig. 62, except that the flagellum was still connected to the spore and the arrangement of the nucleolus in the nucleus could not be ascertained.

When a live healthy zoospore is examined between crossed Nicols, the nuclear cap shows nothing. The materials which make it up are completely isotropic, although the granular surrounding cytoplasm is so diffractive that a sort of shining halo can be seen (Fig. 42). As stated above, this is most likely due to the small size of the particles rather than to any inherent optical activity of their substance. Likewise nothing is revealed in the nuclear cap by dark field; the material seems to be optically empty.

Judging from its disappearance at germination, it seems likely that the cap is a nutritional organelle. It should, then, contain demonstrable food materials, and caps were tested for the common food substances. The iodine test for glycogen as given by Molisch (1923) was negative, the nuclear cap taking on scarcely any deeper hue than the surrounding cytoplasm. While this is not a particularly delicate test, it should give some indication of glycogen if any appreciable quantity were present. A test for reducing sugars was little better, using Trommer's method (Sampson, 1931) of soaking the material in a saturated aqueous solution of $CuSO_4$ and following that with boiling 50% KOH. With this treatment, the nuclear cap, but not the nucleus, became faintly pink, but there were no crystals of copper oxide formed. If there are any reducing sugars in the cap, they are

present in minute quantities, and the color is not conclusive since the same reagents would give a pink reaction with peptides. The fact that Sudan III stains the nuclear cap pale greenish yellow might indicate that some lipoidal material is present, but in comparison with the deep orange yellow assumed by the near-by lipoid droplets, the color of the nuclear cap is not convincing. It is, however, more pronounced than any hue seen in the nucleus or undifferentiated cytoplasm, and it is possible that some fatty compound exists in the cap. Any other possible food material which might be in the nuclear cap would be proteinaceous. Millon's reagent, followed by KOH, causes any protein containing tyrosine to turn orange-red. This test on the nuclear caps was negative. The same result was obtained with nitric acid, which in the xanthoproteic reaction gives a yellow color to proteins containing tryptophane, tyrosine, or phenylalanine. Finally the biuret reaction was tried, with doubtful results. The attempts to demonstrate proteins directly were inconclusive, but it was noted that whenever a strong base was applied to a zoospore, the nuclear cap became greatly swollen. Since the nuclear cap swells in KOH, there must be some reaction which causes a considerable intake of water. A change within the nuclear cap which increases the osmotic value of the contained material could cause such an intake. The reaction of a base such as KOH with proteins containing high percentages of the more acid amino acids could result in the formation of salts which would have a higher osmotic value than the original proteins, and the newly-formed, osmotically active salts would cause an intake of water. Osterhout (1947) has criticised this reasoning as being an over-simplification, but since the nuclear cap does live and grow and is in intimate contact with the nucleus, it is probable, almost certain, on biological grounds that it does contain proteins. Of the protein tests made, the biuret is the only one which is general enough to give a positive result on a wide variety of proteins, and it is unfortunately not very sensitive. The negative results of the protein tests, then, may be due to the small amount of protein present and the insufficient sensitivity and narrow range of the tests.

The acidic nature of the nuclear cap is further brought out by its staining reactions. The stains which have been used successfully to stain this organelle are basic fuchsin, safranine, toluidine blue, methyl green, methylene blue, gentian violet, Janus green, neutral red (which is called neutral because of its hue, not because of its chemical nature) and haematoxylin. It is not a chance happening that all these dyes are basic except haematoxylin, which owes its coloring ability to the formation of a lake and is not to be considered as either acid or basic.

In Flemming's triple stain, the posterior portion of the nucleoli and the nuclear cap, both when in process of condensing and when complete, stain red with safranine. As far as the nuclear cap is concerned, these results are in accord with those of Karling (1937) on *Cladochytrium*.

As information began to accumulate on the composition and structure of the nuclear cap, its intimacy with the nucleus became ever more apparent despite the fact that the cap is physically outside the nuclear membrane. Since iron is known to be a common constituent of nuclei, I thought to find out whether iron could be demonstrated in either the nucleus or the nuclear cap of the *Allo-*

myces zoospore. According to Molisch (1923), minute quantities of "masked" iron can be demonstrated *in situ* in a cell by the Berlin blue reaction. When the zoospores of *Allomyces* were treated with 2% potassium ferrocyanide solution followed by 5% HCl, both nucleus and nuclear cap became blue-green, and were of the same uniform color throughout, so that it was only with difficulty that the nuclear membrane separating the body of the nucleus from the cap could be discerned.

It has been noted earlier that in otherwise untreated material stained with methyl green the nuclear caps were deeply colored just at the last moment of their formation. According to Lee (1928), methyl green is specific for chromatin in the nucleus when used in acidified solution on fresh material. When zoospores are being formed, the nucleus appears in the spore initial only a few seconds before the nuclear cap. If methyl green is added to a sporangium whose nuclei have come into view for the last time and which is therefore on the verge of forming nuclear caps, only the nuclei stain. There is no concentration of dye outside the nucleus. But if the green is added to the sporangium after the deposition of the cap, with its concomitant shrinkage of the nucleus, the cap stains very deeply. Some fundamental change in the composition or physical structure of the cap evidently occurs at the last minute, just at the time when the nucleus is undergoing its reduction in size. Considered alone, this staining reaction would have no great value, as practically all the so-called specific stains will under some conditions stain substances other than the ones they are supposed to stain, but it may have significance in connection with the other reactions.

The Feulgen nucleal reaction for chromatin brings out several definitely stained pink granules in the nuclear cap. This was unexpected because in the first place Hatch (1935) had already tried this reaction on gametes and found the cap completely Feulgen-negative, and furthermore, it is unusual for the reaction to be positive in any cell part outside the nucleus except perhaps in such obviously non-chromatic substances as lignin. At first I suspected that material killed in a Nawaschin-type fixing fluid might give misleading results because if any formaldehyde were not thoroughly washed out of the tissue, it alone would be sufficient to return the leucobase of the dye to its colored state. Since it is impossible to be absolutely certain that all the formaldehyde is washed out of the tissue, the reaction was run on thalli killed with a formaldehyde-free fixative and Figs. 57 and 58 are photomicrographs of Feulgen preparations made from zoospores killed in a Schaudinn-type fixative. Fig. 59 was taken from a Nawaschin-type chrom-acetic-formaldehyde-fixed preparation, and aside from some shrinkage shows essentially the same image. Concerning this plant, Hatch (1935) wrote, "The Feulgen reaction for chromatin differentiates clearly between these two structures (nucleus and nuclear cap). While the nucleus proper gives the characteristic pink-to-red reaction, the nuclear cap does not stain, but stands out a clear white." Concerning the nuclear cap in *Cladochytrium*, Karling (1937) stated, "The use of Feulgen's nuclear stain has not differentiated the cap specifically in our species. In the best of preparations it has not appeared as a

colorless region in contrast to the densely stained chromatin." Karling's statements are negative—the stain has not differentiated—and he neglected to say what did come out; he gave no drawing or photograph of the Feulgen-stained cells; he stated no positive interpretation of the results.

A summary of the tests made on the nuclear cap in this study indicates that the nuclear cap is made to some extent at least of the same substances as the nucleus, but it shows only a part of the picture. It does not show the origin of the cap, which is for the greater part of its bulk formed from a condensation of cytoplasmic granules. This is generally agreed by all investigators. Nor do the data given here show what happens to the cap when the zoospore germinates. After it has escaped from the sporangium and moved about for a time, the spore comes to rest and becomes spherical. As germination commences, the nucleus quits the side of the spore and moves deeper into the middle of it, even while the outline of the nuclear cap remains distinct. Then fat droplets start encroaching upon the nuclear cap, several at a time frequently making temporary indentations in it. The cap gradually shrinks and becomes less and less clearly bounded until, at about the time the first rhizoidal germ tube pushes out, the cap has broken into several small portions and disappeared. I could not determine what became of the substance of the cap, but I did not see it break up into mitochondria, and I did not succeed in following any germination with vitally stained zoospores.

DISCUSSION

One of the major aims of this work has been to learn something about the origin, structure and use of the nuclear cap, and particularly to reinvestigate the relation of the cap to mitochondria. The unusual story of the coalescence of mitochondria to form a nuclear cap led Guilliermond (1941) to say that the origin of the nuclear cap demands verification. The formation of the cap has been described several times in various species of plants, and two explanations have been proposed. Wager (1913) postulated a chromidial origin, and Debaisieux (1919) and Hatch (1935) a mitochondrial one. Karling (1937) and Hillegas (1940) were both more concerned with what the nuclear cap is not than with what it is, and withheld judgment pending further study, while Hovasse (1936) and other investigators have given descriptive accounts of the process of cap formation without venturing any positive guesses as to the source of the material.

In *Polyphagus Euglenae*, Wager concluded that the mass of material collected about the nucleus was chromidial, using Hertwig's definition of chromidia as "discrete chromatin, derived from the nucleus." The chromidia were said to be "extruded from the nucleus . . . for purposes of metabolism and vegetative growth, but . . . at once constituted into nucleus-like structures more or less clearly defined in the cytoplasm."

What we call the nuclear cap Debaisieux called an accessory nucleus. He considered its significance doubtful but thought that "it is formed by the union of mitochondrial chromatic granules, and since in the course of development these arrange themselves in semi-circular peripheral bands it is supposed that they play a part in the formation of the flagellum." The word mitochondria is

mentioned only the one time in the paper, and Debaisieux apparently considered the granules mitochondrial merely because they looked granular and not because of any reactions to special treatment. As Hillegas (1940) pointed out, Debaisieux saw mitochondria without using mitochondrial fixatives. While it is true that sometimes filaments were preserved in *Allomyces* during this study by fixing fluids as acid as pH 1.6, such occurrences were haphazard, while the nuclear caps were always preserved regardless of acidity.

Hatch (1935), in his account of gametogenesis in *Allomyces*, made a detailed and penetrating study of nuclear cap formation. While his description of the events in the gametangia is in agreement with mine for zoosporangia, our interpretations differ. According to Hatch, page 642, "in the formation of the nuclear cap, chondriosomes alone are apparently involved." His chief reason for concluding this is because of the staining reaction of the nuclear cap, about which he wrote "critical coloration . . . is obtained by a combination of Benda fixation with Champy-Kull staining. With this chondriosomal technique the nuclear cap stains blue, the nucleus red." Once more the cap shows its acidic nature in contrast to the relatively basic nucleus, but the fact is that in the Champy-Kull technique mitochondria do not stain blue but red, and if conclusions are to be drawn from staining, the blue nuclear cap would not be mitochondrial. And like Debaisieux, Hatch overlooks the fact that no matter what the material is fixed in, the nuclear caps are preserved.

It should be pointed out that the failure of acid fixers to destroy the nuclear cap does not furnish proof that the cap is not chondriosomal. Plastids are known to be preserved by acid fixers even though plastid primordia are destroyed by any except mitochondrial techniques. Similarly, it might be that the nuclear cap material undergoes some subtle but important change in the young zoosporangium and thereby becomes immune to the dissolving powers of alcohols and acetic acid. If this were the only objection to the mitochondrial origin of the cap, it might be overlooked.

Nuclear caps have been studied critically in *Cladochytrium* by Karling (1937). He noted the shrinkage in size of the nucleoli during the deposition of the cap but decided that "this change is not accompanied by budding or fragmentation and nuclear extrusion as far as I have been able to determine." And concerning the nuclear caps, he says, "In *C. replicatum* I have so far been unable to demonstrate with conclusive certainty and to my own satisfaction that they are chondriosomal in appearance and composition." Karling's results on the Feulgen reaction in the nuclear cap have already been mentioned: he did not find them clearly Feulgen-negative, but neither did he state them to be positive. He did emphasize "the need for more intensive study of the cytoplasmic constituents of the sporangia and zoospores of the lower fungi."

There is one reason for thinking that the nuclear cap is mitochondrial: a series of figures showing the condensation of the particles which make up the cap gives the impression that mitochondria are fusing. But in addition to the objections that the caps stain like nucleoli (and not like mitochondria) and that they are never dissolved by acid fixatives, there is the fact that there is not an unbroken

series from the clearly mitochondrial elements in the hyphae through the development of the sporangia and into the spores. In Hatch's figures, there is an unbridged gap between the filamentous mitochondria of Fig. 24 and the scattered granules of Fig. 25. In that transition period, I could find no mitochondria either in fixed sporangia or in vitally stained sporangia.

The fact that nuclear caps can be stained with Janus green means little. The fluid which fills the exit papillae before dehiscence has a much greater affinity for that dye than do the nuclear caps, and it is well known that non-mitochondrial granules in the islets of Langerhans in the animal pancreas stain vitally with Janus green (Bensley and Bensley, 1938). Further, the nuclear cap will stain as well or better with any basic dye, whether it be neutral red which is supposed to stain vacuoles, or methyl green which is supposed to stain chromatin, or haematoxylin which, given proper pre-treatment and mordanting, will stain practically anything.

A nuclear cap is clearly present and visible without special treatment in the zoospores and gametes of the Blastocladiales, and something similar to a nuclear cap can be brought out by killing and staining the motile cells of certain chytrids. Thus this cell inclusion is found in rather restricted groups of the lower fungi, and so far has been reported nowhere else. There are in other unrelated groups other inclusions which have some superficial resemblances to nuclear caps in that they occur in motile cells, or lie close to the nucleus, or have doubtful significance or are made of unidentified material. Hatch has compared the nuclear cap in *Allomyces* with the so-called limosphere in the sperm of *Polytrichum*, and Wager homologized the chromidial mass in *Polyphagus* with the macronucleus of the Infusoria. There are difficulties in such comparisons that we are not, in our present state of ignorance, ready to meet. The first and greatest difficulty is that the nuclear caps, the chromidial masses, the limospheres and the macronuclei are all problematical bodies. It is not known precisely where they come from, what they are made of, what they do or where they go, and the most detailed studies on them are in considerable disagreement. The futility of trying to compare anything about which there is so little absolute information is obvious. Further, there is a jump across evolutionary boundaries which might be skeptically considered. It is a long way from *Allomyces* to *Polytrichum*, and it would be amazing to find a plant with a simple, coenocytic thallus producing the same unusual cell inclusion as a plant with a "leafy," green, terrestrial gametophyte. There are no intermediate forms to guide us through the intervening void. From *Polyphagus* to *Paramecium* is not such a leap, but even here, the lack of connecting forms between relatively simple zoospores and the ciliates, with probably the most complex cells in the world, makes doubtful the validity of the homology. On more immediate and practical grounds, the nuclear cap and the macronucleus have a serious difference in staining reactions: the cap is stained by the basic dyes, the macronucleus by acid. Aside from producing interesting arguments, such homologies are fruitless and the burden of the proof lies on the promulgator who, lacking means of producing proof, can merely assert.

As nearly as I can conclude from the results of the present study, the nuclear cap is formed partly from condensed material in the cytoplasm and partly from material extruded from the nucleus. There is certainly some cytoplasmic substance included because the volume of the cap is too great to be supplied by the nucleus alone, but there is no positive evidence that there is anything mitochondrial in its make-up. The reasons for thinking the cap is partly nuclear are four. First, the nucleus undergoes a considerable reduction in size precisely at the time when the cap is being formed. Second, the cap and the nucleus are uniformly supplied with iron in small but unmistakable quantity. Third, there are Feulgen-positive particles in the nuclear cap. Fourth, the nucleus and the cap are inseparable even when the spore has all but completely disintegrated.

SUMMARY

1. Zoosporogenesis in *Allomyces arbusculus* has been studied in living and fixed material, making use of direct observation on untreated plants, of vital stains, the centrifuge, polarized light, phase microscopy, lethal staining, cytological staining, the Feulgen technique, and a wide variety of fixing fluids including a Nawaschin-type (CRAF), Lugol's, Schaudinn's, the Zirkle-Erliki fluid, Benda's, Meves's, deRezende-Pinto's, Regaud's, Zirkle's chrome-propionic-nickel, and Zirkle's chrome-formaldehyde-copper.

2. In live material, zoosporogenesis was followed by direct observation. A hyphal tip swells, and is soon separated from the rest of the thallus by a septum, which is laid down centripetally as an irregularly closing diaphragm. After maximum size of the sporangium has been attained, the first indication of spore origins is made. These never become distinct, and soon fade completely, causing the sporangium to go into a period of apparent quiescence, during which nothing can be seen but lipoid droplets, many of them appearing in circles outlining the nuclei. This disappearance stage lasts for some time, even several days under some conditions, but terminates with the second appearance of the spore origins. When these appear, nuclei move to the surface of the sporangium, lipoid droplets collect between the places occupied by the nuclei. Cleavage fissures cut out polyhedral blocks of protoplasm, and about each nucleus, within a minute or two, a nuclear cap collects. The exit papillae, numbering from one to half a dozen, become distended, and when the spores are at last completed, one papilla ruptures and the spores begin to escape. The other papillae temporarily subside, but soon open. The first spores to emerge remain massed for a few moments before swimming away; the spores which come out later move off individually, swimming by means of the flagella after a time of ameboid movement.

3. The sporangium increases in size until it reaches its maximum just before the first appearance of the spore initials, after which there is a sudden sharp decrease in volume. A second even sharper decrease occurs just before the release of the spores, while the cleavage fissures are cutting out spore origins for the final delimitation of the spores.

4. Methods of purifying the vital stains neutral red and Janus green are de-

scribed. The optima of pH and concentration for neutral red were at pH 7±, and 0.0002% (1:500,000). The optima for Janus green were pH 7 ± and 0.001% (1:100,000). The hyphal mitochondria stained well, but none were found in the maturing sporangia. The specificity of the dye in staining nuclear caps is doubtful.

5. In fixed material, zoosporogenesis was followed after the usual cytological and after mitochondrial fixations. Cleavage progresses centrifugally, and spores are delimited before the nuclear caps are deposited. The mitochondria, which are conspicuous in living hyphae, are preserved by many killing fluids, even those containing acetic acid and being, in the case of the Nawaschin-type fixer used, as acid as pH 1.6. The mitochondria disappear in the young sporangium. When the nuclear caps are about to be formed, deeply staining granules appear in the cytoplasm, and these have been interpreted as mitochondria by some investigators. The granules flow together forming ever larger masses until finally one somewhat crescent-shaped body becomes inseparably applied to the nucleus.

6. Microchemical tests, polarized light, and staining reactions gave information on the composition of parts of the thallus and zoospores. The walls of the hyphae gave tests only for chitin. Although the walls are anisotropic, anisotropism was also demonstrated in insect chitin. There is probably pectic material in the walls of the resistant sporangia. The lipoid droplets contain true fats, as shown by their isotropism and their saponification reactions, but their lack of solubility in some fat solvents indicates the presence of some unidentified substance which is not fatty.

7. The centrifuge shows that the nuclei are high, the lipoid droplets low, and the cytoplasm medium in specific gravity. Mitochondria were never discovered in centrifuged material. In the zoospore, the contents formed in layers, which contained, from lightest to heaviest, the lipoid droplets, the cytoplasm, the nucleus, with the nucleolus at its heavy side, the nuclear cap, and a thin line of unidentified granules.

8. A side body was found in the zoospores from resistant sporangia and also in zoosporangium zoospores.

9. A significant reduction in the size of nuclei, occurring when the nuclear caps are formed, is compared with the general increase in size which occurs when reproductive nuclei of species without nuclear caps are formed.

10. The flagellum is a tubular, faintly anisotropic organelle whose fine structure is fibrillar. It is probably resorbed at germination.

11. The nuclear cap contains iron in about the same quantity as the nucleus. It may contain small amounts of fatty material and reducing sugars, but no glycogen. Protein tests were generally negative, but other considerations make it likely that proteins are present. The Feulgen reaction shows positive granules in the cap.

12. The conclusion, based on staining reactions, that the nuclear cap has a mitochondrial origin is illogical, since the nuclear cap is acid in its reaction and always stains with basic dyes, while, in the technique upon which the mito-

chondrial theory of the nuclear cap was based, the mitochondria stain with the acid dye. If the nuclear cap were mitochondrial, it should stain like mitochondria.

13. The nuclear cap material is never dissolved by acid killing fluids, acetic acid, or alcohols, as it probably would be if it were mitochondrial.

14. Although separated from the nucleus by the nuclear membrane, the nuclear cap can not be physically removed from the nucleus, regardless of treatment. The two bodies never part until the zoospore germinates, at which time the nuclear cap disintegrates. Its precise fate is unknown; it has not been observed breaking up into mitochondria.

15. It is concluded that the nuclear cap is composed partly of cytoplasmic and partly of nuclear material. It is too large to be derived entirely from the nucleus, yet it is formed precisely at the time when the nucleus is shrinking; it shares a positive iron content with the nucleus; it is never separated from the nucleus; it contains Feulgen-positive granules.

Note: There has been received too late for review here a paper by Sparrow and Hammond (Amer. Jour. Bot. **34**: 439–445, 1947) in which the authors demonstrate desoxyribose nucleic acid in the cytoplasm of microsporocytes of several spermatophytes, by means of ultra-violet absorption spectra and the Feulgen technique.

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EXPLANATION OF PLATES

All figures are of *Allomyces arbusculus* unless otherwise indicated. Drawn figures were made with camera lucida, arm at 120 mm. and mirror at 45°, with paper at table level. Figs. 1–27: final magnification after reduction for reproduction 1130 × unless otherwise stated; all figures except Fig. 27 from living, untreated material. Explanation of abbreviations: mc, mitochondrion; n, nucleus; nc, nuclear cap; no, nucleolus; sb, side body; v, vacuole; wl, whip-lash.

PLATE 22

Fig. 1. End of a hypha showing inclusions: mitochondria, nuclei, nucleoli, and lipoid droplets, × 1525.

Fig. 2. 1:55 P.M. Hyphal tip swelling, cytoplasm and inclusions moving into apex.

Fig. 3. 2:10 P.M. Young sporangium with newly-formed septum and thick mass of lipoid droplets almost obscuring nuclei. No mitochondria visible at this time.

Fig. 4. 2:25 P.M. Young sporangium beginning to take on mature shape. Nuclei but no nucleoli visible; mitochondria can be seen in basal portion of sporangium where lipoid drops are lacking.

Fig. 5. 3:30 P.M. Sporangium reaches full size; septum finally as thick as sporangial wall. No nuclei, nucleoli, or mitochondria to be seen, only lipoid drops, thicker about periphery. First swelling of an emergence papilla.

Fig. 6. 3:35 P.M. Maturing sporangium. Hints of nuclei appearing, vague and transitory. Nothing else visible.

Fig. 7. 3:45 P.M. Maturing sporangium. Two more exit papillae appear. Central region, poor in lipoid droplets, sends out bays into peripheral region which is rich in droplets. Optical section.

Fig. 8. 3:52 P.M. Maturing sporangium. Temporary view of nuclear regions has disappeared. A fourth exit papilla appears. Central vacuole ill-defined; nothing visible but lipoid droplets.

Fig. 9. 4:25 P.M. Maturing sporangium. Few rings of lipoid droplets surrounding nuclei; central vacuole larger and more distinct. Central vacuole drawn in optical section; nuclei drawn in surface view.

PLATE 23

Fig. 10. 5:40 P.M. Mature sporangium, with zoospores forming. Nuclei with nucleoli suddenly evident, with greatest density of lipoid droplets in regions between nuclei. Some nucleoli eccentric, others centric.

Fig. 11. 5:43 P.M. Zoospores forming. Thin clear regions forming between spore initials, making splits in the masses of lipoid droplets; the clear splits, of different refractivity from rest of the cytoplasm, separate the spore initials into polyhedral masses. The nucleoli are commonly eccentric by now, and the clear area beside the nucleus shows the condensation of the nuclear cap. The lipoid droplets are being forced into the peripheral cytoplasm of the spore initials. Flagella still not visible.

Fig. 12. 5:46 P.M. Exit of zoospores, with those outside the sporangium still congregated in a mass.

Fig. 13. 5:55 P.M. Only a few zoospores left. One in escaping has the flagellum pulled taut, with its posterior end caught in the spore mass which is still in the sporangium.

Fig. 14. Zoospore motionless but alive. Whip-lash at end of cilium can be seen without staining. × 1875.

Fig. 15. Small vesicle appears at end of flagellum. × 1875.

Fig. 16. Vesicle enlarges as flagellum shortens. Two granules visible at periphery of vesicle, and two connecting strands passing over its surface. × 1875.

Fig. 17. Two smaller vesicles grow on original one. × 1875.

Fig. 18. Ectoplasm disintegrates suddenly, letting lipoid droplets and vacuoles float away in the water. The nucleus is swollen and the nuclear cap vacuolate. The flagellum is irregularly twisted. Nucleolus of two portions: an anterior dark and a posterior refractive part. Surface view, on up-focus, showing relation of nuclear cap to nucleus. × 1875.

Fig. 19. Nucleus disintegrates explosively, swinging remains of spore and separating flagellum from body of spore.

Fig. 20. Flagellum becomes ravelled; vacuole with surrounding tonoplast becomes irregular in shape and floats away. Nuclear cap becomes vague and seems to be dissolving; only nucleolus remains as clear as ever.

Fig. 21. Portion of a hypha at the base of a developing sporangium just before the formation of the septum.

Fig. 22. A few seconds later than Fig. 21. Surface view showing continuous line produced by the developing septum.

Fig. 23. Optical mid-section of hypha, drawn as soon after Fig. 22 as possible, showing that the septum develops centripetally. Although no substance is visible in the central region of the septal area, some hindrance to the passage of cytoplasmic inclusions is evident from the accumulation of lipoid droplets below the incipient septum.

Fig. 24. Optical section about half-way between a surface view and a median optical section, drawn as soon after Fig. 23 as possible, showing the irregular manner in which the material of the septum is being laid down.

Fig. 25. Median optical section showing newly-formed septum, much thinner than sporangial wall, with clear cytoplasm above and below.

Fig. 26. Septal region drawn about ten minutes after Fig. 25. The septum has bulged toward the sporangium, and become thicker about the periphery.

Fig. 27. Zoospore treated with nitric acid, with resulting splitting of flagellum. Drawn by Mr. Paul Titman.

PLATE 24

Fig. 28. Living, unstained hypha as seen with phase microscope. Lipoid droplets and mitochondria visible, wall out of focus. × 424.

Fig. 29. Filamentous mitochondria in Zirkle-Erliki preparation. × 815.

Fig. 30. Centrifuged hypha, killed in osmic acid. No stain. Lipoid droplets at top, nuclei at bottom, no mitochondria. × 815.

Fig. 31. Mitochondria in CRAF fixation. Compare with Figs. 33 and 34. × 1530.

Fig. 32. Portion of rather old vegetative hypha killed in chrome-formaldehyde-copper. Large, empty vacuoles. × 2160.

Fig. 33. Portion of a hypha killed in Zirkle-Erliki's fluid. Mitochondria and lipoid droplets clear, nuclei poorly preserved. × 1630.

Fig. 34. Mitochondria preserved by acid fixing fluid: Nawaschin type (CRAF) at pH 1.6. × 1260.

Fig. 35. Young sporangium, probably in early disappearance stage. Rather large, scattered vacuoles. Chrome-formaldehyde-copper. × 900.

Fig. 36. Cross section of a sporangium with nuclear caps in process of condensing. Double nature of nucleoli visible, the darker part in the photograph representing the red-stained portion, and the lighter the blue. CRAF, Flemming's triple stain. × 1260.

Fig. 37. Cleavage fissures separating spore initials while the nuclear cap is condensing. Chrome-formaldehyde-copper. × 900.

Fig. 38. Sporangium during period of circlets of lipoid drops, showing peripheral displacement of nuclei and rather empty central region. de Rezende-Pinto's. × 900.

PLATE 25

Fig. 39. A chain of three empty sporangia of *Allomyces* photographed in polarized light. The extinction positions are particularly marked in the round middle sporangium and in the septa. × 685.

Fig. 40. Oogonia, oospheres, and hyphae of *Achlya* sp., a form with cellulose walls, photographed in polarized light. The heavy walls are strongly anisotropic. × 245.

Fig. 41. A crystal of chitosan sulfate prepared from the walls of *Allomyces*. Polarized light. × 1080.

Fig. 42. A zygote with the two flagella twisted about one another, and showing the anisotropic property of the flagella. See text for explanation of apparent anisotropism in body of cell. Osmic acid, unstained. × 3190.

Fig. 43. Zoospore with side body, nucleus, nuclear cap. Lugol's. × 1980.

Fig. 44. A row of crystals resulting from the saponification of the lipoid droplets in a hypha. Polarized light. × 2230.

Fig. 45. Sporangia centrifuged one hour at 5200 × gravity, fixed in osmic acid, unstained. Lipoid droplets at upper side, layer of granular material near but not on lower side. No nuclei, no mitochondria. × 815.

Fig. 46. Sporangium in disappearance stage. Cytoplasm granular, but without any filamentous mitochondria. Zirkle-Erliki. × 900.

Fig. 47. A surface view of a zoosporangium after being centrifuged for one hour at 5200 × gravity, showing circles of lipoid droplets adhering to nuclei. Nuclei are scattered through the lower half. Nucleoli visible. The dark spot near the top is an exit papilla. Osmic acid. × 1300.

Fig. 48. Femur of Cecidomyiidae as seen between crossed Nicols. The tibia would be seen at the right side of the picture if it were not in the extinction position. × 415.

Fig. 49. The hypha to which the sporangium in Fig. 46 was attached. A few filaments can be seen in the hypha as contrasted with the sporangium which has none. Zirkle-Erliki. × 900.

Fig. 50. A resistant sporangium undergoing dehiscence, showing along the vertical cleft how the wall splits on a preformed line. Living material. × 760.

PLATE 26

Fig. 51. Surface view of sporangium during stage of circles of lipoid droplets. Living material. × 1560.

Fig. 52. Small portion of a sectioned sporangium killed in Schaudinn's fluid during early stage of deposition of nuclear cap material. Cleavage is well advanced, but the cap material is in the form of minute scattered granules in the cytoplasm. × 2445.

Fig. 53. Section of a sporangium with nuclear caps slightly more advanced than in Fig. 52. Granules have begun to coalesce, but are still scattered throughout cytoplasm. Schaudinn's. × 1385.

Fig. 54. Sporangium with nuclear caps more advanced than in Fig. 53. The cap material is in blobs, and has begun to collect about the nuclei. Schaudinn's. × 1385.

Fig. 55. Nuclear caps just a little earlier than those in Fig. 56 being completely condensed, but not having assumed their final form. Schaudinn's. × 2445.

Fig. 56. Nuclear caps complete. Zoospores still in the sporangium but nucleoli and nuclear caps are so much darker than sporangium wall and cytoplasm that the latter could not be printed out of the photographic negative without obscuring the relation of the nucleus, the nucleolus and the nuclear cap. Schaudinn's. × 2685.

Fig. 57. Zoospores stained with Feulgen technique. Feulgen-positive granules in cap, nucleus darkly stained. Schaudinn's. × 2250.

Fig. 58. Zoospores stained with Feulgen technique. Cytoplasm invisible, nucleus and cap stained. Schaudinn's. × 2250.

Fig. 59. Sporangium containing zoospores stained with Feulgen technique. CRAF fixation. × 2200.

Fig. 60. Zoospore in ameboid condition, showing distorted nuclear cap. Chrome-formaldehyde-copper. × 1980.

Fig. 61. Heterogeneous appearance of nuclear cap when fixed in Regaud's fixative. × 1890.

Fig. 62. Zoospore centrifuged one hour at 5200 × gravity. From top to bottom: lipoid droplets, cytoplasm, nucleus with nucleolus at heavy side, nuclear cap, unidentified granules. Osmic acid. × 2200.

Fig. 63. Mature zoospores. The vacuolate condition of the nuclear cap can be seen in the spore on the lower right. Chrome-formaldehyde-copper. × 1980.

Fig. 64. Zoospores exposed to show dark, swollen nucleolus with unstained posterior portion to which the flagellum is attached. Chrome-propionic-nickel. × 2160.

Fig. 65. Zoospores similar to those in Fig. 64, exposed to show flagellum and cytoplasm. Chrome-propionic-nickel. × 2160.

PLATE 22

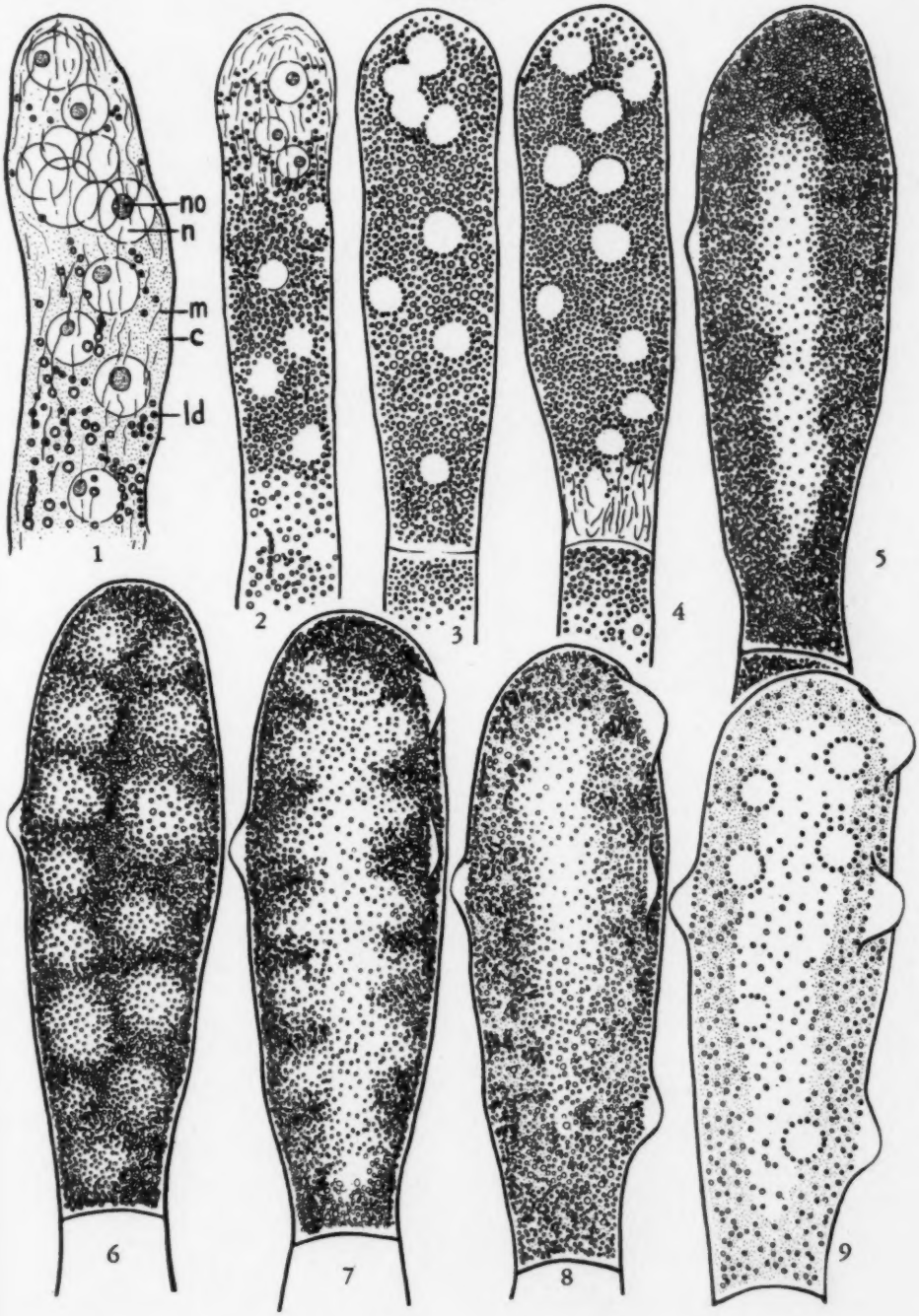


PLATE 23

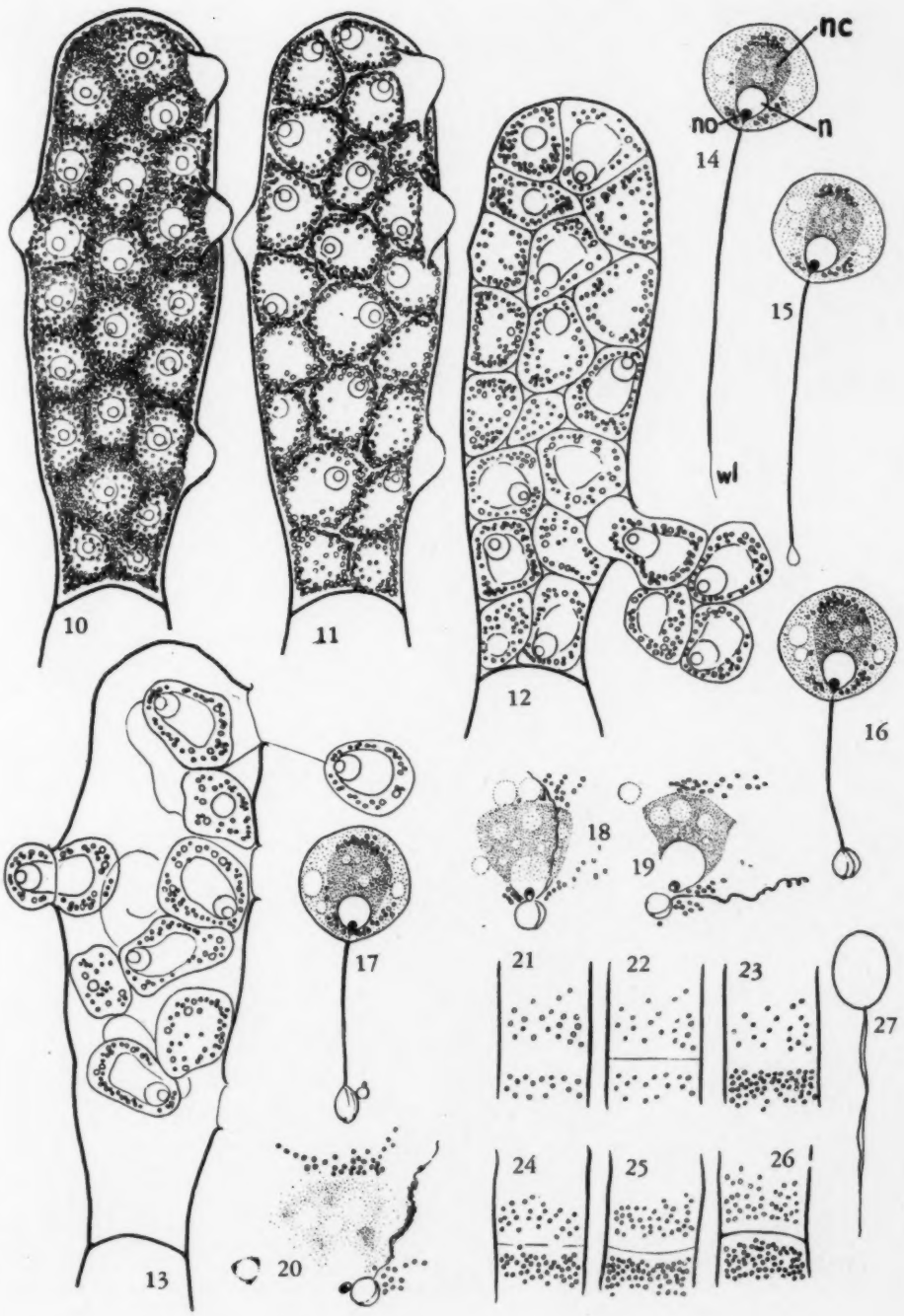


PLATE 24

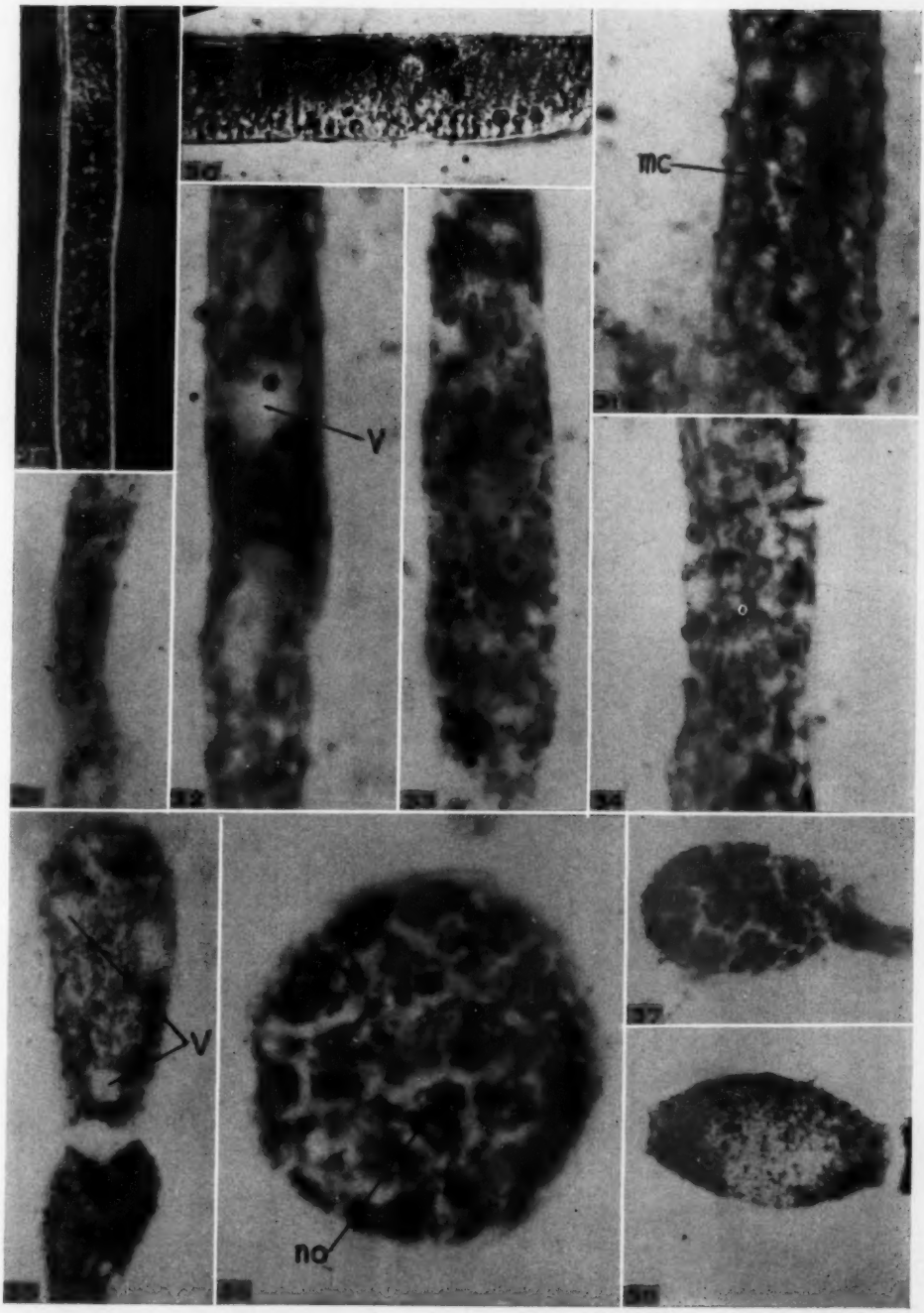


PLATE 25

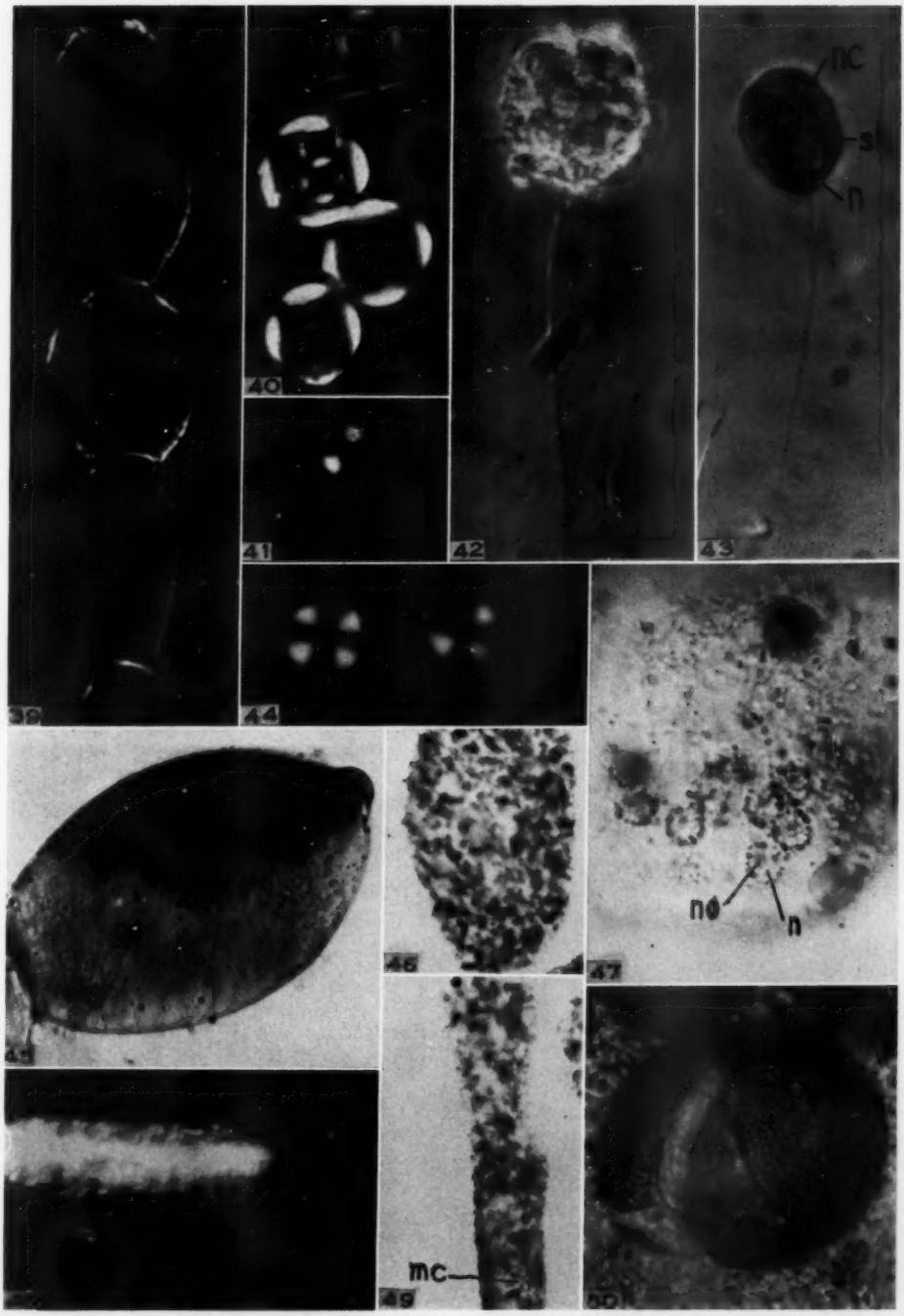
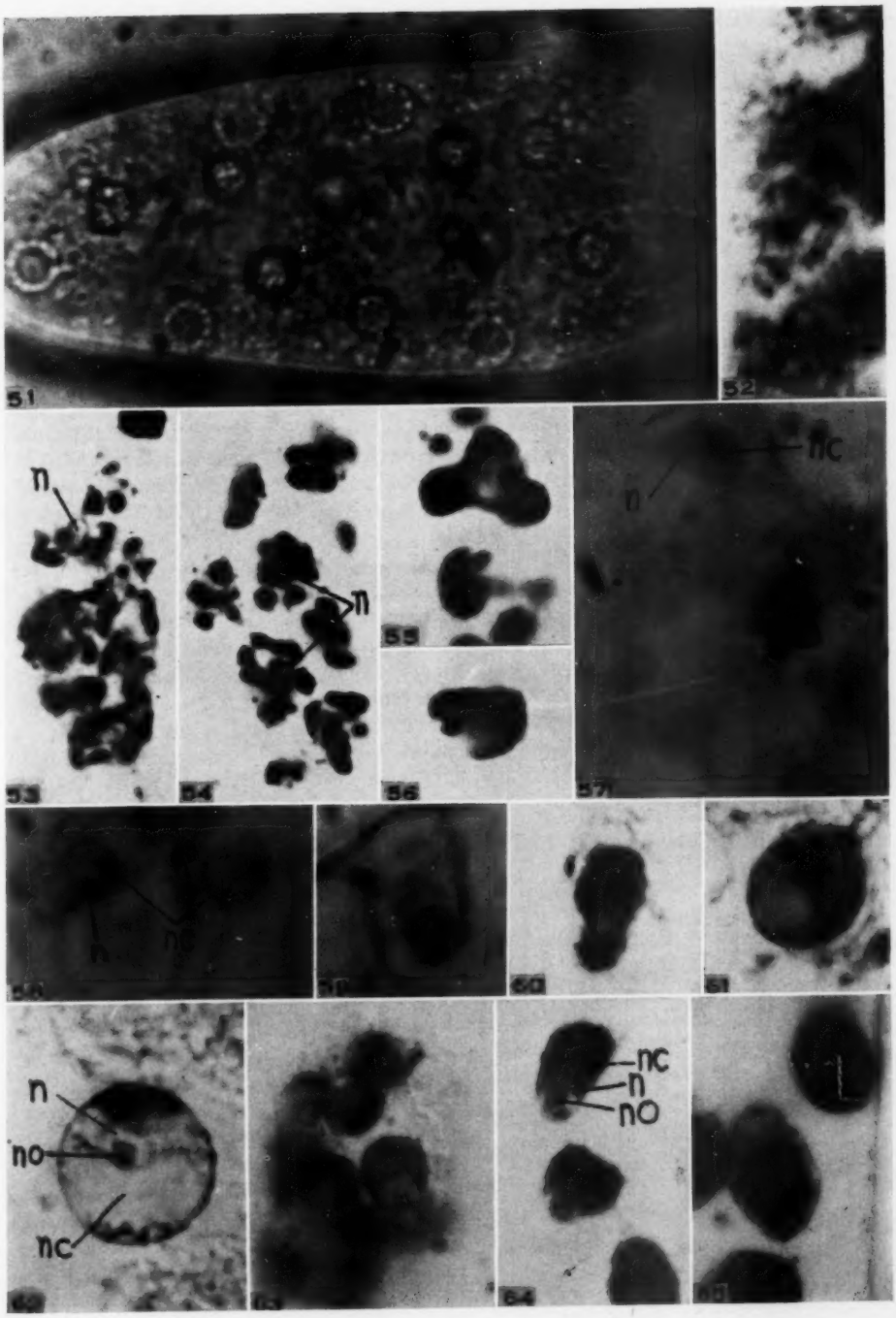


PLATE 26



A RARE SPECIES OF ISOACHLYA FOUND IN NORTH CAROLINA

By Arthur Kelman

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Plate 27

A species of *Isoachlya* not previously reported from North America was found recently in the course of an examination of soil samples for water molds. This fungus possesses several morphological characteristics which serve to distinguish it from the other common species. However, after a study of the fungus and a review of the literature, it was found to be in close agreement in most respects with the brief description of *Isoachlya Itoana* Nagai (1931). A more complete description of a very similar form was reported as a new member of the genus *Isoachlya* by Dissmann in the same year. Apparently neither of these men was cognizant of the other's work. In order to clarify possible synonymy of species, a description of the morphology and growth habits of this water mold is presented and a comparison is made with similar forms described in Japan and Germany.

The soil sample in which the fungus was found was obtained from beneath a cypress tree on the campus at Chapel Hill on February 9, 1946. Sterile water was added to a small quantity of soil in a Petri dish. Three days after halved boiled hemp seeds were placed in the suspension, a delicate white weft of mycelium had developed at the outer edge of the seeds. After transfer to separate Petri dishes, the cultures were examined and in addition to several common soil-inhabiting water molds, a form differing from other Chapel Hill species was observed. This was isolated in pure culture by making single hyphal tip transfers. The cultures obtained served as a source for all subsequent material during the course of the study.

Mycelial development on potato-dextrose agar plates is very rapid and at room temperature a white cottony mycelium will spread over an entire plate in 4 days. Sexual and asexual reproductive structures do not usually form in potato-dextrose agar. Gemmae will form in 2% plain agar after 14 days and in some cases scattered single oogonia are found. A circular mycelial mat forms rapidly on hemp seed in tap water cultures and reaches a maximum diameter of about 18 to 20 mm. in 12 to 14 days. Mycelial development on boiled corn seed is not as rapid as on hemp seed. It is quite typical for oogonial formation to be initiated on the 3rd day of growth on hemp seed. At the end of 7 days the culture has a granular appearance due to the large numbers of oogonia which are produced.

The vegetative hyphae are rather typical for the genus, measuring about 28–30 microns at the base, tapering at the upper end to 10–12 microns in width. The branching is not very abundant in vigorously growing cultures.

Primary zoosporangia are formed at the tips of the vegetative hyphae. They are elongated and cylindrical in shape, tapering to a rounded tip at which a distinct discharge papilla (10–16 microns long) usually develops. Formation of sporangia occurs during the early phases of growth and as soon as oogonia begin to form the number of new sporangia produced decreases rapidly. Secondary sporangia are typically formed by lateral branching, whereas internal proliferation which is characteristic of some of the other members of the genus is rarely found. Basipetal formation of secondary sporangia may occur and in these forms the zoospores are released through a lateral exit pore (fig. 4). The sporangia are 21–52 microns wide, mostly 28–35 microns, and are quite variable in length, ranging from 161 to 560 microns, mostly 300–390 microns. The abundance of sporangia in cultures on hemp seed is favorably affected by placing small portions of boiled oak leaves in the plates.

The zoospores when mature usually escape rapidly from the sporangium. However, sluggish discharge is quite frequent. The zoospores (8–11 microns in length) are diplanetic and biflagellate and swim as usual in the genus. The period of zoospore activity is quite variable, ranging from 15 minutes to several hours, following which encystment takes place. Frequently several zoospores are found encysted within a discharged zoosporangium. Clustering of the zoospores in *Achlya*-fashion at the mouth of the sporangium does not occur.

In hemp seed cultures, gemmae are formed in moderate abundance soon after rapid vegetative growth has ceased. The gemmae have dense granular contents and exhibit great variation in size and shape. Some are globular (56–105 microns in diameter), borne on short thick stalks, while others are borne at the ends of long hyphae. Single elongated club-like gemmae, expanded in the center or at the upper end, are more common than globular or oval forms found in chains.

Profuse and rapid development of oogonia with numerous antheridia is one of the more striking features of this species. The rather small oogonia (32–57 microns, mostly about 43 microns in diameter) are formed in a racemose fashion on short lateral stalks and in clusters near the ends of branches. The oogonia are almost completely spherical with a short basal neck and a distinct smooth hyaline wall which stains a dark reddish-purple with zinc chloriodide. There is no evidence of pits in the wall. Occasionally short, rounded, blunt projections are noted on the distal surface. In cultures in which sporangial development is not abundant oogonia may be found at the ends of main hyphal branches. Intercalary oogonia, with a somewhat elongated atypical shape, are very rarely found. On no occasion were oogonia found developing in chains.

Clavate antheridia, which may branch at the end in a finger-like fashion, normally develop in a cluster on the stalk below the oogonium. The number of antheridia which develop is greatly influenced by the type of medium in which growth occurs, the most profuse and rapid development taking place in sterile distilled water cultures on hemp seed. The number formed will vary between 5 and 12. When boiled oak leaves are placed in these cultures, antheridia do not form so rapidly and the number will rarely exceed three on a single stalk.

The manner of attachment of the antheridium to the oogonium varies. The tip may be appressed at the base, side or upper surface, or the end may be attached for some distance along the entire side of the oogonial wall. Although the species is characterized by the antheridia arising from the oogonial stalk, they may arise from the main hypha when the oogonial stalk is very short. An antheridium may attach itself to a neighboring oogonium wall in the same cluster if the two stalks are very close together, but this is an unusual development. Diclinous antheridia are very rarely found. Usually after several weeks the antheridia have disintegrated so that no signs of them are left on the oogonial walls.

The mature oospore almost completely fills the oogonium. Within the hyaline oospore wall (1.5–2.5 microns thick) there are two layers of oil globules which surround the dark, dense central portion of the egg. Single oospores were found in 89%, two oospores in 8.6%, and three oospores in 2% of the oogonia examined. Oogonia have been found with four oospores and in several instances an oogonium with five oospores was noted. Germination of the oospore has not been observed.

In the description of *Isoachlya Itoana* presented by Nagai emphasis is placed on the androgynous antheridial branches, single oospores, and cymose branching of the zoosporangia. The measurements listed for zoosporangia are generally smaller than those which are found in the species isolated at Chapel Hill, whereas measurements for oogonia and oospores are in substantial agreement.

Although Nagai's description is not too complete and contains few illustrations, on the basis of the diagnosis presented there is not sufficient evidence to warrant placing the form found in North Carolina in a new species. In the same year that Nagai's work was published, a new species, *Isoachlya subterranea*, was reported by Dissmann from Germany. A rather detailed description of the morphology and growth habits of the fungus was presented and comparison was made with the other species previously described. This present species differs from Dissmann's form in the less abundant internal proliferation of zoosporangia, presence of smooth instead of irregularly striate or finely roughened oogonial walls, and in having at times more than two eggs in the oogonium. In other respects there is a striking similarity, in particular, the formation of androgynous antheridia, size and shape of oogonia and oospores and their general arrangement. It is felt that the differences between *Isoachlya Itoana* and *Isoachlya subterranea* are probably not great enough to consider them separate species. However, without making direct comparison with the original cultures it is unwise to reduce Dissmann's species to synonymy.

Isoachlya Itoana can be readily distinguished from other members of the genus. In the following species, *Isoachlya unispora* Coker and Couch, *I. eccentrica* Coker, and *I. monilifera* (DeBary) Kauffman, antheridia have not been demonstrated (1923, 1937). The species with antheridia include *I. toruloides* Kauffman and Coker, *I. intermedia* (Coker and Harvey), and *I. anisospora* (DeBary) Coker. Diclinous antheridia are commonly not present on more than half of the oogonia in *I. toruloides* and the number of eggs varies from 1–6, with occasionally as many as 8–12; *I. anisospora* has numerous eggs, 1–20, mostly 2–6, and diclinous

antheridia; *I. intermedia* differs in respect to elongate, irregular, tapering sporangia and the formation of antheridia which arise immediately beneath the oogonium, usually one or rarely two to an oogonium. Another addition to the genus which has not been reported from North America was made by Richter (1936–37). He described a new species, *Isoachlya terrestris*, isolated from soil in Germany. This differs from *I. Itoana* in the development of antheridial branches further from the oogonium than the oogonial diameter, the presence of 1–5, mostly 2–3, oospores in the oogonium and the eccentric fat globule in the mature oospore. *Isoachlya Itoana* is close to *Saprolegnia megasperma* Coker, in some respects, but the two species can easily be distinguished.

Isoachlya Itoana Nagai

Mycelial development moderately vigorous, hyphae slightly branched, 28–30 microns at the base, tapering to 10–12 microns in width at the upper end; zoosporangia elongated, cylindrical, 21–52 microns, mostly 28–35 microns, wide and 161–560 microns, mostly 300–390 microns, long, tapering to a rounded tip, with a distinct discharge papilla always present; secondary sporangia formed by lateral branching, internal proliferation very rare; zoospores diplanetic, biflagellate, escaping from apical pore of sporangium; sluggish discharge frequent; gemmae variable in shape, ranging from elongated club-shaped forms to globular spherical types, the latter borne singly or less frequently in chains; oogonia spherical, 32–57 microns, mostly about 43 microns, in diameter, formed in great abundance on short lateral branches, never in chains; wall smooth, hyaline, staining reddish-purple with zinc chloriodide, without pits; antheridia always present, arising from oogonial stalk, the number varying with the type of medium, clavate, occasionally branching slightly at the tip in a finger-like fashion; oospores usually single, varying 1–3, rarely 4, centric, spherical, 25–42 microns, mostly 32–36 microns, in diameter, with a thick hyaline wall (2–3 microns) when fully mature; germination not observed.

SUMMARY

A rare species of *Isoachlya*, which is characterized by oogonia with single oospores predominating and androgynous antheridia, is described. Comparison is made with very similar types reported as separate species from Japan (*Isoachlya Itoana* Nagai) and Germany (*Isoachlya subterranea* Dissmann). The fungus is distinct from forms previously reported from North America, and is considered to be identical with *Isoachlya Itoana* Nagai.

The author is indebted to Dr. John N. Couch of the Department of Botany, University of North Carolina, for his kind assistance.

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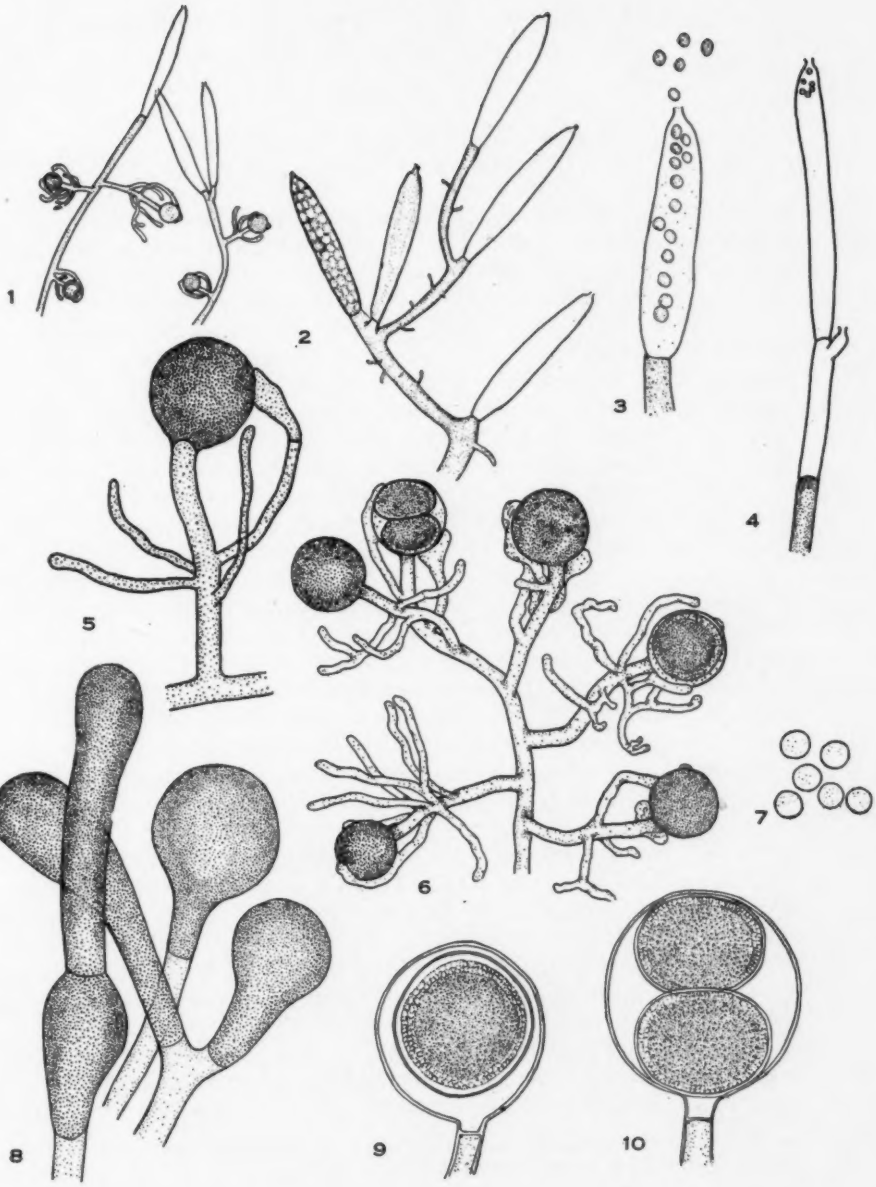
EXPLANATION OF PLATE 27

Isoachlya Itoana

Fig. 1. Hyphae with typical zoosporangia, oogonia, and antheridia. × 85.
Fig. 2. Cluster of zoosporangia. × 175.
Fig. 3. Zoosporangium with zoospores emerging. × 370.
Fig. 4. Basipetal arrangement of zoosporangia, one with several zoospores encysted at the mouth, the other with lateral discharge papilla. × 220.
Fig. 5. Immature oogonium with androgynous antheridia. × 385.
Fig. 6. Cluster of oogonia with antheridia. × 220.
Fig. 7. Encysted zoospores. × 530.
Fig. 8. Gemmae. × 220.
Fig. 9. Typical mature oospore within oogonium, antheridia degenerated. × 530.
Fig. 10. Mature oogonium with two oospores. × 530.

All drawings were made with Spencer camera lucida.

PLATE 27



OBSERVATIONS ON GONIDIA FORMATION IN CYLINDROSPERMUM*

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It seems to be the opinion among many phycologists that mature filaments of *Cylindrospermum* always form gonidia. A few statements from some of the leading phycologists indicate the prevalence of this idea. Smith (1933, p. 92) makes this statement in regard to spore formation in *Cylindrospermum:* "Unlike *Anabaena*, one cannot find filaments that do not show at least early stages of akinete formation." Fritsch (1945, p. 808) says: ". . . in *Cylindrospermum* and *Gloeotrichia* it [spore formation] appears always to ensue when the trichomes have reached a certain size." Geitler (1932, p. 812) is not so positive in his statement but does infer that mature filaments always have gonidia. He says: "In typischer Ausbildung ist *Cylindrospermum* immer leicht kenntlich. Jugendformen ohne oder mit unreifen Dauerzellen können mit *Anabaena* verwechselt werden." Many other authors mention the difficulty of distinguishing "young forms" of *Cylindrospermum* from *Anabaena*, and of identifying young forms with immature spores as to species.

During the winter, spring, and summer of 1947 an extensive study of the Myxophyceae of North Carolina was undertaken. In the course of this investigation one thousand six hundred forty-two collections were made from eighty-three different counties. *Cylindrospermum* was a common form frequently collected. In a goodly number of these specimens, however, no evidence of spore formation was found. This fact attracted the attention of the author and caused him to examine and study carefully some of these collections of *Cylindrospermum* which did not show gonidia. The results of the study of some of these are given below.

1. In January 1947 an extensive growth of *Cylindrospermum* was observed on rocks and soil along the banks of a small stream near the Wake Forest College Stadium. The growth was characteristic of the genus and appeared to be in excellent condition. A small rock which was covered with the alga was taken into the laboratory and the plants examined carefully. The trichomes and the heterocysts fitted the description of *C. minutum*, but no gonidia were to be found. The rock was placed in a culture dish and kept in the laboratory until March. During this time the specimen was examined almost daily but no evidence of gonidia formation was ever noted, even though the plant spread over the entire surface of the dish and appeared in excellent vegetative condition. The original

* This investigation and a study of the Myxophyceae of North Carolina were aided by research grants from the Carnegie Foundation for the Advancement of Teaching, Research Fellowship from the University of North Carolina, the Margaret Cannon Howell Fellowship, Highlands Biological Laboratory and a Research grant from the A. A. A. S.

growth was kept under observation, and frequent examinations were made to see if spores were forming. On March 5th—about three months after the growth was first discovered—some filaments showed young gonidia. On March 10th it was difficult to find any trichome which did not have a gonidium. Study of the mature spores confirmed the identification as *C. minutum*.

2. On June 18th a patch of *Cylindrospermum* about the size of a man's hand was discovered growing on a clay bank of a small stream flowing out of the golf course pond at Wake Forest College. Upon microscopic examination, it too, was found to be void of spores. The vegetative growth and heterocysts fitted the description of *C. catenatum*. On June 26th it still appeared to be in prime condition and was spreading on the bank, but there was no indication whatsoever of gonidia. On July 5th the plant mass appeared yellowish brown and the trichomes were evidently disintegrating. No gonidia were ever found and the identification is still in doubt.

3. The third growth of *Cylindrospermum* collected without gonidia was taken from a healthy growth in a drain ditch in Perquimans County. No spores were found when the plants were first examined nor did any develop in the laboratory. This appeared to be *C. muscicola*.

4. On July 26th and 27th three specimens of *Cylindrospermum* which did not possess gonidia were collected in Stanly, Union, and Moore Counties. All of these came from rich growths which appeared in prime condition.

5. On August 18th an extensive growth of what appeared to be *C. stagnale* was collected from the mud on the bottom of the stream flowing from the golf course pond at Highlands, N. C. No signs of gonidia were found in this collection. A second collection was made from the same place on August 22nd, but there were still no gonidia present. A little farther down the same stream and growing in a similar habitat *C. stagnale* was collected on August 22nd. This was producing an abundance of spores.

From the above observations it appears that *Cylindrospermum* may and does develop mature trichomes and grow for a considerable length of time without forming gonidia. This lack of spore formation is not limited to any one locality or habitat, nor is it confined to any one species of the genus.

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BREEDING HABITS OF A CAVE SPIDER, NESTICUS CARTERI EMERTON

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Two Text Figures

Though there has been some difference of opinion as to the exact scientific name of the spider considered in this paper the name of the spider used is *Nesticus carteri* Emerton as determined by B. J. Kaston, 1945.

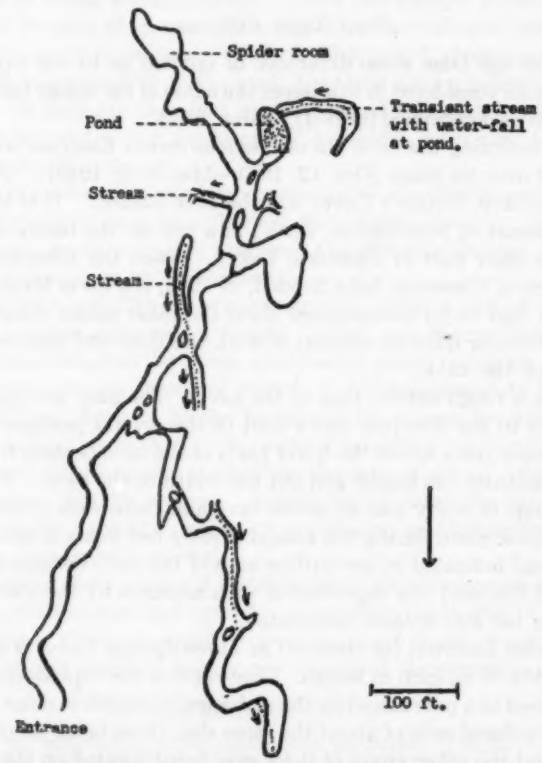
The data concerning the cave life of *Nesticus carteri* Emerton were obtained for a period of over six years (Oct. 12, 1933—March 19, 1940). Three Springs Cave (Delap's Cave, Buttry's Cave) was the cave studied. It is located about ten miles northeast of Morristown, Tenn., in a cliff on the banks of the former Holston River (now part of Cherokee Lake). When the Cherokee Dam was built the waters of Cherokee Lake flooded, in 1942, the lower levels of the cave and the study had to be discontinued since the inner spider room became inaccessible. However quite an amount of work was done and data secured before the flooding of the cave.

Fig. 1 shows a rough outline map of the cave. The map was made to give a general idea as to the direction and extent of the several passages of the cave. It was made some years before the lower parts of the cave became flooded. The scale in feet indicates the length and not the width of the cave. Three Springs Cave at the time of study was an active cave and there were quite pronounced changes that took place during the comparatively few years of study. For example, the pond indicated in the outline map of the cave became entirely filled with sand and the sand was deposited in such amounts by the transient stream that the water fall also became obliterated.

Nesticus carteri Emerton (as observed in Three Springs Cave) is a small spider about one eighth of an inch in length. The color of the cephalothorax and legs may be described as a pale tan while the abdomen is grayish in color. The spider has six pearly colored eyes of about the same size, three being on one side of the head region and the other group of three eyes being located on the other side of the head region. There are also two small dark median eyes in front and between the two other eye groups. It has, like other spiders, four pairs of legs and a pair of pedipalps in front which resemble another pair of legs, especially so in the female spider. The spider spins a small web of only a few strands of spider silk. The cocoons or egg cases of *Nesticus carteri* are carried by the female spider by means of a small thread attached to the posterior part of the abdomen. The number of eggs contained in an egg case probably ranges from 15 to 20. (On April 22, 1935, one female spider was collected that was carrying an egg case which contained 17 little spiderlings which had not yet emerged

from their cocoon.) The length of time a *Nesticus carteri* female carries her egg case is probably somewhat over a month under cave conditions.

The outstanding physical condition under which the spider lived was that of absolute darkness. Another characteristic ecological condition was the high relative humidity of the spider room, which was always above 90%, as well as the uniform temperature of the cave which in the spider room was practically 55 degrees F. year in and year out.

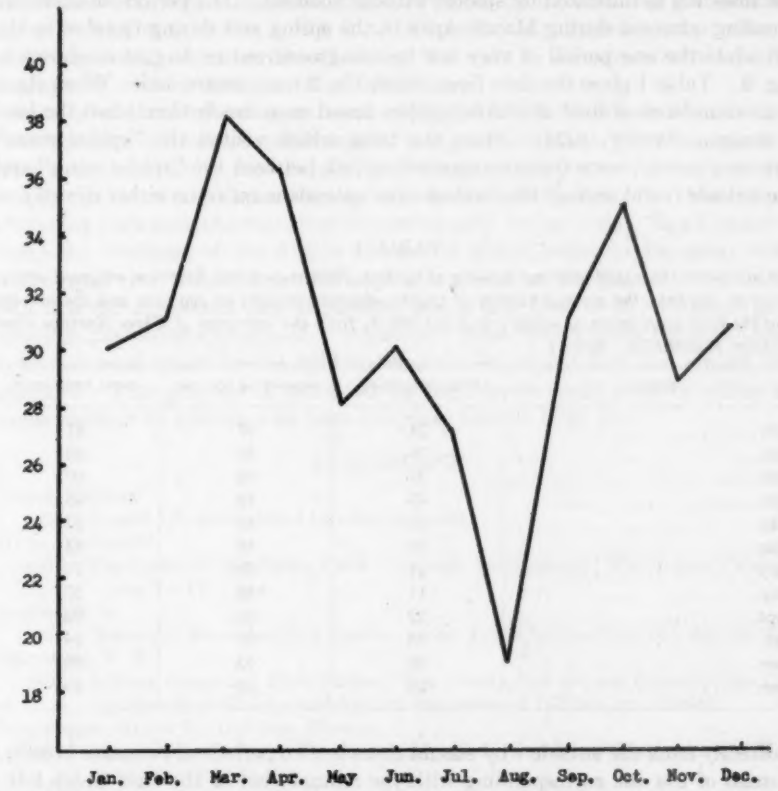


TEXT FIGURE 1. Three Springs Cave (Delap's Cave, Buttry's Cave)

The animal co-inhabitants of the spider room with *Nesticus carteri* were collected, for the most part, in meat traps from Jan. 29th to Feb. 19th, 1940 (Barber, 1931). There were found to be present millipeds (especially *Cambala annulata*), rove beetles (*Echochara lucifuga*), fungus gnats (*Sciara* sp.), small flies (*Leptocera cellularis*), bats, numerous collembola and small mites. The bats made a yearly visit to the spider room in August of each year.

The breeding habits of *Nesticus carteri* are rather interesting and in a way somewhat unexpected. The female spiders attach their cocoons or egg cases

to the posterior part of their abdomen and carry them thus until the little spiderlings come out and "shift for themselves." By observing the spiders with their cocoons, which make the appearance of the spiders quite conspicuous, one may easily determine the time of breeding by counting the number of cocoons for each month during the year. This was done for six consecutive years;



TEXT FIGURE 2. The ordinates represent the percentage of spiders carrying an egg case among the total spider population. The abscissas represent the month of the year in which the observations were made.

that is, once a month a visit was made to the "spider room" and the number of female spiders carrying egg cases or cocoons was counted and a record made of each count in a certain limited area. Also a count was made of the number of spiders without cocoons in the same area. The same limited area was used each year to make the spider counts comparable for all of the years in which the spider was studied. The results for the entire six years of study showed that at least one or more spiders bred each month throughout the entire period. However

there were certain months when more spiders were breeding, as indicated by their attached cocoons, than there were in other months. One might readily surmise that there should be at least one high breeding period during each year but instead of that the spiders showed two periods of excessive breeding as indicated by the number of spiders carrying cocoons and only one period of very low breeding as indicated by spiders without cocoons. The periods of excessive breeding occurred during March–April in the spring and during October in the fall while the one period of very low breeding occurred in August as shown in Fig. 2. Table 1 gives the data from which Fig. 2 was constructed. When there is an abundance of food available, spiders breed more freely than when the food is meager (Savory, 1928). Since the bats, which visited the "spider room" but once yearly, were the only connecting link between the "spider room" and the outside world and all the food of cave animals must come either directly or

TABLE 1

The six year (1934–1939) average number of spiders, Nesticus carteri Emerton, observed carrying an egg case, the average number of spiders observed without an egg case and the average of the total population of spiders at about 800 ft. from the entrance of Three Springs Cave (near Russellville, Tenn.)

MONTH	WITH AN EGG CASE	WITHOUT AN EGG CASE	TOTAL POPULATION
Jan.	24	57	81
Feb.	26	59	85
Mar.	34	56	90
Apr.	35	63	98
May	23	59	82
June	25	58	83
July	21	58	79
Aug.	11	46	57
Sept.	22	50	72
Oct.	33	61	94
Nov.	26	63	89
Dec.	26	59	85

indirectly from the outside why should there be two periods of excessive breeding instead of the one corresponding with the annual visit of the bats which left a supply of fresh bat guano each year? That the time of egg-laying of *Nesticus carteri* Emerton was correlated with the annual visit of bats in August is indicated by the conditions observed after the bats failed to visit the cave in 1939, 1940, and 1941. After 1939 the number of spiders carrying cocoons in October decreased to 5 in October, 1940, and to 4 in October, 1941. The waters of Cherokee Lake were rising so rapidly to flood the cave that a count of the spiders was made Feb. 28, 1942, instead of waiting to make the count in March–April which was one of the high breeding periods. However there were found only 5 spiders carrying cocoons on Feb. 28, 1942.

At the time of their yearly August visit to the "spider room" the bats came in considerable numbers and roosted on the roof of the cave at practically the

identical place year after year. Fresh bat guano was dropped on the old mound of bat guano in such abundance as to cover completely the old mound of guano and the amount was so great as to cover up even all evidence of spiders and their slight webs which were on the old guano before the bats came. This yearly visit of the bats and the destruction of all spiders on the guano mound, due to the excessive deposit of fresh bat guano, accounts for the one very low breeding period in August as shown in Fig. 2, as well as for the very low total number of spiders present. However the spiders on the walls of the cave after the yearly August visit of the bats appeared about as usual.

Soon after the fresh bat guano was deposited by the bats certain excrement flies, *Leptocera cellularis*, began to appear in some abundance. Their appearance apparently furnished a food supply for the spiders on the nearby wall, at any rate by October the spiders evidently had or were getting enough food to sustain a breeding peak each October after the bat's yearly August visit. In a number of weeks the freshness of the August deposit of guano began to disappear while at the same time a fungus growth began to appear on the guano. Coincident with this the excrement flies diminished and a new kind of fly, a fungus gnat *Sciara* sp., made its appearance. Apparently these fungus gnats helped at least to furnish food again for the spiders since the excrement flies had almost disappeared. This perhaps is indicated by the March–April peak in which an excess number of spiders were seen carrying cocoons (Fig. 2).

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NORTH CAROLINA SPECIES OF VOLVARIA*

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Plates 28–32

KEY TO THE SPECIES

Cap large, white, beautifully silky tomentose all over; growing on wood, usually rotting places on standing trees, etc. ..... *V. bombycina*
Cap not as above
  Growing on the ground or in trash
    Spores over 8 μ long
      Cap very viscid or glutinous
        Cap up to 8 or 10 cm. broad
          Cap white or pallid flesh color, margin not striate ..... *V. speciosa*
          Cap dusky gray or brown, margin striate ..... *V. gloiocephala*
        Cap 3–4.5 cm. broad ..... *V. Earlei*
      Cap only moderately viscid, or hardly so, brown, not striate ..... *V. floridana*
    Spores less than 8.5 μ long
      Cap about 6–10 cm. broad, drab to buffy brown ..... *V. volvacea*
      Cap much smaller
        Stem glabrous; volva white, glabrous
          Cap strongly umbonate, distinctly striate ..... *V. umbonata*
          Cap not umbonate, faintly striatulate ..... *V. pusilla*
        Stem pubescent
          Volva white, long and narrow ..... *V. pubescentipes*
          Volva dark brown, short and wide, densely matted-pubescent ..... *V. cinerea*
  Growing as a parasite on *Clitocybe nebularis* ..... *V. Loweana*

Volvaria bombycina (Schaeff.) Fr. Syst. Myc. **1:** 277, 1821; Hymen. Eur., p. 182. 1874.

Agaricus bombycinus Schaeff. Fung. Bavar. **4:** Ind. 42. 1774.
Volvaria bombycina Quél. Champ. Jura et Vosges **1:** 80. 1872.
Volvariopsis bombycina (Schaeff.) Murr. Mycologia **3:** 281. 1911.

Plate 28 and pl. 32, fig. 1

Cap large to very large, hemispheric to convex, white or creamy white, turning to tan with lavender tints, particularly on margin, when old; surface covered all over with short, silky hairs that distinguish the plant at once; the margin at first floccose and extending beyond the gills, often beautifully crenulate when fresh. Flesh pure white and soft, only 2.5 mm. thick half way to the margin.

Gills free, broad, about 12 mm. in center, moderately close, white at first then turning flesh color, in age deep flesh color.

Stem pure white, very smooth and shining, tapering upward, arising from or near the base of a large, ample, persistent volva.

Spores between russet and tawny (Ridgway), oval, smooth, 4–5.5 x 6–8.5μ.

* Two of the species here included (*V. umbonata* and *V. Loweana*) have not yet been found in this state but are to be expected here.

This is one of our most beautiful mushrooms, usually growing from decaying places on trunks of oak, maple or other deciduous trees, but also found on fallen trunks and stumps and has been collected on sawdust. It is edible and widely distributed but is not at all common. Kauffman gives it as reaching a width of 13.5 cm. in Michigan and Massee found one 14 inches broad in England.

Illustrations: Atkinson. Mushrooms, fig. 134 (fig. 137 of 1901 ed.). 1900.
Bresadola. Icon. Myc., pl. 526.
Cooke. Ill. Brit. Fungi, pl. 293.
Gillet. Champ. Fr., Hymén., pl. 710 (253).
Güssow and Odell. Mushrooms and Toadstools, pl. 62, fig. 1.
Hard. Mushrooms, figs. 191–193. 1908.
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Kauffman. Agar. Mich., pl. 100.
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Lloyd. Comp. Volvae U. S., fig. 8. 1898.
McIlvaine. Amer. Fungi, pl. 59 and 59a. 1900.
Patouillard. Tab. Anal., fig. 330.
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Smith. Mycologia **37:** p. 441. 1945.

North Carolina. Chapel Hill. No. 197. On rotting wood, Oct. 10, 1902. No. 1161. On trunk of paper mulberry (*Broussonetia*), July, 1914. Spores (print) 4–5 x 6–7.6μ. No. 1581. In a rotting place in side of a *Quercus nigra* trunk, June 26, 1915. Spores (print) 4.8–5.5 x 7–8.5μ. This plant was remarkable in having a long stem with the volva attached around the middle of it. No. 9713. In a hollow in a silver maple tree on campus, June 11, 1934. No. 10276. On a dead place in a living poplar tree, Sept. 10, 1936. No. 11045. On a lombardy poplar in the arboretum, Sept. 20, 1938.

Volvaria speciosa Fr. Syst. Myc. **1:** 277. 1821.
Amanita speciosa Fr. Obs. Myc. **2:** 1. 1818.
Volvaria speciosa Gill. Champ. Fr., p. 388. 1876.
Volvariopsis speciosa (Fr.) Murr. North Amer. Fl. **10:** 143. 1917.

Plate 30 (bottom) and pl. 32, fig. 2

Cap about 5–8 (10) cm. broad, nearly plane, or slightly gibbous, often campanulate, smooth, shining and viscid, nearly white or a pallid grayish flesh color, the center slightly darker, not striate. Flesh very thin on marginal half, up to 8 mm. thick in center, soft, not brittle, white, taste and odor of uncured peanuts (decidedly so and noticed by several persons).

Gills white when young, turning flesh color and reddish flesh in age (cinnamon-rufous of Ridgway); free, crowded, about 5 mm. deep, the edges uneven and eroded, and usually whitish.

Stem tapering upward, about 8–12 mm. thick at cap, smooth except on base which is tomentose, when very fresh, the tomentum quickly collapsing; shining, white or cream, brown when touched, bulbous, the bulb up to 2.5-3 cm. thick; flesh crisp, solid, white, soft. Volva rather thick and firm, small for the size of the plant, strongly attached to most of the bulb, free edge narrow and unevenly torn, usually close to stem; outer surface tomentose and largely covered with earth.

Spores (of No. 1475) rusty cinnamon (between cinnamon-rufous and hazel,

Ridg.), smooth, ellipsoid, 7.4–8.5 x 13–15.5μ. Cystidia, according to Ricken, are "vesicular-pyriform, 60–70 x 20–36μ, sometimes pointed."

The plant is much like *Pluteus cervinus* in size, shape, and color of gills. It is widely distributed but apparently rare in America except in the west, where it is said to be common. It is found in cultivated places, preferring rich garden soil. Curtis reports it from North Carolina and Ravenel from South Carolina. *Volvaria speciosa* is said by some to be poisonous but in America it has been pronounced harmless and good. McClatchie says it is abundant in California and is "a fine edible agaric." Mr. John Dearness of London, Ontario, reports the eating of *V. speciosa* by a family for two weeks or more with no ill effects, and says he himself has eaten it and found it without doubt edible. He thinks its reputation for being poisonous is based on its confusion with *V. gloiocephala*.

Illustrations: Boudier. Icon. Myc. **1**: pl. 84.
Bresadola. Funghi Mang., pl. 44; Icon. Myc., pl. 528.
Cooke. Ill. Brit. Fungi, pl. 297.
Cordier. Champ., pl. 6, fig. 1 (as *A. speciosus*).
Gillet. Champ. Fr., Hymén., pl. 714(255).
Güssow and Odell. Mushrooms and Toadstools, pl. 62, fig. 2.
Krieger. Nat. Geog. Mag. **37**: pl. 5. 1920.
Patouillard. Tab. Anal., fig. 640.
Ricken. Blatterpilze **2**: pl. 70, fig. 1.
Rolland. Atl. Champ., pl. 55, fig. 123.

North Carolina. Chapel Hill. No. 1475. In sandy garden soil under a grape vine, Nov. 14, 1914. Two plants. No. 1476. Same spot, Nov. 16, 1914. Spores 6.8–8.2 x 11.2–14μ. No. 4312. In rich soil by cedar hedge in the arboretum, May 10, 1920. Spores 7–9.3 x 11.5–15μ.

Volvaria gloiocephala (DC) Fr. Syst. Mycol. **1**: 278. 1821.

We have not found this species but Curtis reports it from North Carolina. It is considered rare. Kauffman finds it in Michigan and it has been reported from several other middle and western states. In the Curtis-Berkeley manuscript (copy in the New York Botanical Garden Library) we find:

No. 2897. (*Ag. gloiocephalus* DC.)

Cap 3–4 in. wide, glabrous, viscous, livid or pale lead color, with large angular fragments of the volva adhering, margin striate. Substance fleshy, white, tasteless. Lam. whitish, becoming discolored by the reddish ferruginous spores, edges denticulate lacerate, ventricose free. Stipe 3–5 in. long, solid, white, fibrillose, with a satiny lustre, separable from the cap, base bulbous, margin of the volva loose or sometimes obsolete. Dec. April.—In cultivated lands.

The species is much like *speciosa* in form and color (usually darker) and is distinguished from it by striate margin, usually darker color, volva fragments on cap, and lack of cystidia. Also the spores as given by Kauffman (6–7.5 x 11–13 μ) and by Rea (7 x 12 μ) are decidedly smaller. (Murrill gives the spores as 9 x 19 μ.) The species is said to be highly poisonous. Bresadola's plate does not show cap as striate, but text says "margin at length striate."

Illustrations: Bresadola. Funghi Mang., pl. 45; Icon. Myc., pl. 529.
Cooke. Ill. Brit. Fungi, pl. 298.

Dufour. Atl. Champ., pl. 32, fig. 73. 1891.
Gillet. Champ. Fr., Hymén., pl. 711(254).
Juillard-Hartmann. Icon. Champ. Sup., pl. 73, fig. 7.
Patouillard. Tab. Anal., fig. 224.
Ricken. Blatterpilze **2:** pl. 70, fig. 2.
Rolland. Atl. Champ., pl. 56, fig. 124.
Note: Berkeley's illustration labelled *V. speciosa* in Outl. Brit. Fung., pl. 7, fig. 3, looks more like *V. gloiocephala*.

Volvaria Earlei Murr. Mycologia **4:** 332. 1912.
Volvariopsis Earlei Murr. Mycologia **3:** 282. 1911.

Plate 29 (bottom, right) and pl. 32, figs. 3–6

Cap 3–4.5 cm. broad, evenly convex of subumbonate, glabrous, nearly pure white, viscid when wet, shining when dry, not striate. Flesh soft, white, about 4 mm. thick at stem, odor when quite fresh like that of *speciosa*, but faint after maturity.

Gills close, ventricose, about 4 mm. wide, broader toward stem than toward margin of cap, edges finely eroded, free and later seceding farther from the flaring top of the stem, color pallid white, then through flesh color to darker rusty-brown.

Stem about 3.5–5.7 cm. long, 3–5 mm. thick near cap, tapering upward from a scarcely enlarged base (except where fused with the small volva), white, solid, glabrous; flesh whitish but usually translucent, firmer than in cap. Volva very short, hardly 1 cm. long when quite fresh, soon fading, only the upper half or less free, thin, about 0.5-1 mm. thick when fresh, splitting into 2 or more lobes which flare upward at first but usually soon collapse against the stem, surface softly felted with a silky appearance, not really pubescent.

Spores (of No. 13175, print) rusty pink, about cinnamon to cinnamon-rufous (Ridg.), smooth, oval-ellipsoid, 6.3–8.5(9.3) x 10.5–14.4μ. Basidia stout-clavate, 4-spored, 11–13.7μ thick, about 35μ long. Cystidia not at all abundant and so delicate as to collapse easily and be difficult to find, very thin-walled, projecting about as far as the basidia, about 18.5μ thick, pointed or rounded and with or without a thickened cap on the end.

A cap just breaking through the volva was 12.7 mm. broad, silky, subshining, quite glabrous, nearly spherical, slightly subgibbous, nearly white with very pale drab tint, center slightly more tinted. This same little plant a day later showed the stem not even pubescent but with an appressed silky-fibrous surface.

Our plants have been compared with the type collection of *V. Earlei* from Cuba, now in the New York Botanical Garden, and found to be close duplicates in structure and spores (type spores measuring 6.8–8(9) x 11.5–14 μ). The types grew in a banana field in Cuba, and so far as we have found the species has not been reported since the original description.

This little plant is near *V. speciosa*, which it resembles in the viscid, nearly white, non-striate cap, odor of raw vegetables (Irish potato, peanut, etc.), narrow volva rim, and in the spores. It differs in much smaller size, very smooth, nearly glabrous volva (see drawing of No. 13175), and in the entirely glabrous stem which is scarcely enlarged below. In the dried plants the stem bases with volva are remarkably small, in strong contrast to the large bulbous bases of *speciosa*.

North Carolina. Chapel Hill. No. 2360, July 4, 1916. No. 3351, June 12, 1919. Spores 7.4–8 x 11–14.8μ. No. 13170, Sept. 15, 1942. No. 13175, Sept. 17, 1942. All the above collections were taken from the vegetable garden of W. C. Coker,—two from a pile of rotting grass clippings, the others from earth near it. No. 13980. On bare earth in a corn patch about 100 yards from the location of the above numbers, Aug. 14, 1946. Spores(print) 7–8.5 x 10.5–14.8μ.

Volvaria floridana Murrill. Mycologia **30:** 371. 1938.
Volvariopsis floridana Murrill. Mycologia **30:** 368. 1938.

Plate 32, figs. 7, 8

In 1918 we found in pure sharp sand on a dune on Smith's Island, N. C., one small plant of a *Volvaria* that we could not place. Since seeing a plant of the type collection of *V. floridana*, kindly sent by Dr. Murrill, we now think our plant is that species and are so entering it with some question. We describe our plant as follows:

One plant; cap brownish, 4.5 cm. broad, hemispheric, then expanded, smooth, somewhat viscid, not striate, thinly covered nearly all over with sand grains, one patch of volva near margin; flesh whitish, toughish, about 5 mm. thick at stem, taste flattish, odor mild.

Gills free about 7 mm. wide, close but hardly crowded, pallid white, then rosy.

Stem 5.5 cm. long, 9 mm. thick, white, smooth, glabrous, slightly viscid, solid, somewhat enlarged below for about 2 cm. by the white *volva* which is fused with the stem except for a short free rim with torn margin, the volva surface sparsely fibrous and holding sand rather loosely, the grains easily falling off and leaving the surface minutely pitted.

Spores rosy, smooth, oval-ellipsoid, 7.4–9.5 x 12.5–16.6 (17.7)μ. Cystidia not seen.

Differs from *speciosa* in glabrous stem, less viscid cap, and volva patches on cap; from *gloiocephala* in less viscid and non-striate cap; from *V. Earlei* in less viscid cap and larger volva; and from all of these in larger spores. In all critical characters our plant seems in agreement with *V. floridana*, and the very large spores, larger than in any other species we have seen, are exactly alike (see drawings). Our plant is smaller but that may be due to its growth in pure sand. Murrill considers *floridana* as perhaps only a variety of his *alabamensis*, a plant that we have not found. It is to be noted that the vegetation of Smith's Island, N. C., with its tall palmettos is very like that of northern Florida. Murrill says that his plants were avoided by squirrels, which he took as a warning against eating them.

North Carolina. Smith's Island, Brunswick County. In bare, pure sand on top of a sand dune, April 6, 1918.

Volvaria volvacea Bull. Herb. Fr., pl. 262.

Plate 31 and pl. 32, fig. 9

Cap 6–10 cm. broad, ovate then expanded and depressed around the the center, even, regular; surface dry, distinctly marked with inherent radial fibers and often radially cracked superficially, color about drab brown to buffy brown, darkest in center, the cracks lighter. Flesh about 5 mm. thick in center, membranous on

the marginal third, very soft but toughish, white with a tint of the cap color; taste flattish and rather peculiar, odor meaty-aromatic.

Gills not crowded, rounded and quite free from the stem, up to 11 mm. wide, very thin and papery, deep flesh color, about onion skin pink.

Stem 4.5–6.5 cm. long, slender, minutely furfuraceous, color of cap, lightly stuffed then hollow, enlarged below and set in a large brownish, delicately floccose volva which may be clean or discolored with earth.

Spores (of No. 3319) about onion skin pink, oval, smooth, pointed at one end, 4–5 x 5.5–6.3μ. For cystidia, basidia, etc., see Hesler (l.c.).

This is a rare plant with us. It is found in gardens, greenhouses, cellars, etc., in the eastern United States and Europe.

Illustrations: Bulliard. Herb. Fr., pl. 262.
Cooke. Ill. Brit. Fungi, pl. 294.
Flora Danica, pl. 1731, fig. 2.
Hesler. Journ. Tenn. Acad. Sci. **16:** No. 1: fig. 7. 1941.
Juillard-Hartmann. Icon. Champ. Sup., pl. 74, fig. 1.
Lloyd. Comp. Volvae U. S., fig. 9, 1898.
Massee. Brit. Fungi and Lichens, pl. 15, fig. 2. 1911.
McIlvaine. Amer. Fungi, pl. 60. 1902.
Patouillard. Tab. Anal., fig. 331.
Rolland. Atl. Champ., pl. 55, fig. 122. This is a very pale form.
Sowerby. Engl. Fungi, pl. 1.

North Carolina. Chapel Hill No. 2259. Under shrubs, June 26, 1916. No. 3319. Same spot as above, June 7, 1919.
Schweinitz. Syn. Fung. Car. No. 541.

Florida. Gainesville. Murrill, coll. (U.N.C. Herb.) Spores ovoid-ellipsoid, 4–5.9 x 6.6–9.5μ, larger than in ours but plants otherwise typical. Rea (England) gives spores 4–5 x 6–7μ.

Volvaria umbonata Peck. Bull. Torr. Bot. Club **26:** 64. 1899.

We have not found this species but it is reported from Ohio, Minnesota, Missouri, and more recently from Tennessee by Hesler (Journ. Tenn. Acad. Sci. **18:** 297. 1943). It is said by Hard to be quite common on lawns at Chillicothe, Ohio (Mushrooms, p. 241, fig. 194. 1908). His description agrees very well with Peck's except that he says the stem is hollow. His good photograph is the only illustration we have seen. The type plants were sent to Peck from Cincinnati by Lloyd, who says it usually grows in lawns (Myc. Notes, No. **2:** 10. 1899). Peck's description follows.

Pileus thin, campanulate, becoming convex or nearly plane, prominently umbonate, distinctly striate, slightly viscid when moist, silky when dry, white; lamellae moderately close, free, not extending beyond the margin of the pileus, pale flesh color; stem equal or slightly thickened at the base, glabrous, solid, white, the ruptured membranous white volva persistent, irregularly split or lobed on the margin and forming a shallow cup at the base of the stem; spores broadly elliptic, uninucleate, variable in size, 5–7μ long, 4–5μ broad.

Pileus 2–3 cm. broad; stem 5–6 cm. long, about 4 mm. thick.

Lawns and grassy places, Ohio, Lloyd.

The species is most closely allied to *Volvaria media* (Schum.) Fr., from which it is distinguished by its larger spores and the striate margin of the pileus. In our plant the striations sometimes extend to the umbo.

Dr. Stevenson has kindly sent us two collections of unnamed Volvarias from the Lloyd Herbarium in Washington. One of these, to judge from the descriptions, could easily be *V. umbonata*. The dried caps are buffy clay color, about 18 mm. broad, very strongly umbonate, and several of them are striate; surface entirely glabrous, not shining but inherently fibrous; gills moderately close, about dull cinnamon color, rather narrow (1.5 mm. dry); stems entirely glabrous, color of cap but slightly more brown, about 3.5–5.5 cm. long and 1.5–2 mm. thick in the center, slightly enlarged downward to the base, the very base somewhat flaring and surrounded by the rather short (up to 5 mm. long) flaps of the dull brown volva; spores oval, smooth, 3.8–4.8 x 4.8–6.7(7) μ.

These unnamed plants are from Ohio and as there is no collection labelled *V. umbonata* in the Lloyd Herbarium it is almost certain that these are the plants that Lloyd described as Peck's *umbonata* (Myc. Notes No. **2:** 10. 1899). They may be a part of the type collection sent to Peck.

Volvaria pusilla (Pers.) Fr. Syst. Myc. **1:** 279. 1821.
Amanita pusilla Pers. Obs. Myc. **2:** 36. 1799.
Agaricus parvulus Weinm. Hymen. Imp. Ross., p. 238. 1836.
Volvaria parvula Quél. Champ. Jura et. Vosges **1:** 81. 1872.
Volvariopsis pusilla (Pers.) Murr. N. Amer. Fl. **10:** 141. 1917.

Plate 29 (top) and pl. 32, figs. 10, 11

Cap up to 9 mm. broad in our plants, hemispheric, then convex-expanded, not umbonate, dull white or pale grayish, silky-fibrillose, the fibers partially free, particularly toward the margin, not viscid, margin striatulate, becoming crenate for a short distance in age or incipient drying.

Gills pinkish at maturity, moderately close, adnate to a swollen disk around the top of the stem, up to 1 mm. wide, ventricose, edges even.

Stem up to 8 mm. long, slender, 1 mm. thick, shining white, glabrous, solid. Volva lobed, color of cap but glabrous, short but ample and entirely free from the stem and seated on a white pad on top of the soil.

Spores salmon, smooth, oval-elliptic, 3.7–4.8 x 5.5–7.4μ. Basidia 7–9μ thick, 4-spored, the sterigmata very short. Cystidia ten-pin-shaped, up to 14μ thick and projecting about 33μ.

The dry cap is dull and has a spongy-felted appearance. The cap is described as white or grayish white by different authors and the stem as solid or fistulose. It may be a little larger than in our specimens: Kauffman says 12 mm.; Murrill, 15 mm.; Konrad and Maublanc, even up to 3 cm.

The species is widely distributed in eastern America and has been reported from North Carolina by Schweinitz, Curtis, and Beardslee and from Tennessee by Hesler. It is the smallest of the Volvarias and is distinguished by its small size, white or grayish white color, glabrous stem and glabrous white volva which is entirely free from the stem, a character which is clearly shown in the figures of Hard, Kauffman, and Ricken, and is conspicuous in our plants although not obvious in the photograph. The cap is variously described as umbonate, obtuse or slightly depressed. In our plants the cap is evenly convex. Fries, who considered *pusilla* and *parvula* the same, referred to Bulliard's plate 330 (as *A. volvaceus minor*) in his treatment of both, but if those figures are

correct his plant differs from ours in having the lower half of the volva fused with the stem.

Illustrations: Boudier. Icon. Myc. 1: pl. 86 (as *V. parvula*).
Bresadola. Icon. Myc., pl. 533 (as *V. parvula*). 1929.
Clement. Minnesota Mushrooms, fig. 31 (as *V. parvula* and exactly the same photograph which appears in Hard as *V. pusilla*). 1910.
Cordier. Champignons, pl. 6, fig. 2 (as *A. parvulus*). 1870.
Gillet. Champ. Fr., Hymén., pl. 713 (256).
Hard. Mushrooms, fig. 195. 1908.
Kauffman. Agar. Mich., pl. 101 (top). 1918.
Konrad and Maublanc. Icon. Sel. Fung., pl. 18, fig. 1.
Lloyd. Myc. Notes No. 4; fig. 1. 1899.
Patouillard. Tab. Anal., No. 332 (as *A. parvulus*). 1883.
Persoon. Obs. Myc. 2: pl. 4, fig. 4. 1799.
Ricken. Blatterpilze 2: pl. 70, fig. 3. 1915.

North Carolina. Chapel Hill. No. 7193. In a shady, mossy garden bed with a film of blue-green algae, Oct. 10, 1923. No. 7345. In damp sandy soil under cedar, July 7, 1924.

Volvaria pubescentipes Pk. Rept. N. Y. St. Mus. **29:** 39, pl. 1, figs. 1–3. 1878.

Plate 32, figs. 12–15

Cap 1.5 cm. broad, convex, umbonate, mostly white but streaked with cream and with umbo cream color, surface slightly tomentose, not viscid, not striate but with outer fibrous layer cracking radially. Flesh thin, white; odor sweet, taste mealy.

Gills free, creamy flesh color, 2.2 mm. wide, moderately close, the edges whitened and very minutely fimbriated by the cystidia.

Stem 4 cm. long, 1.4 mm. thick, equal, white, finely and evenly tomentose all over. Volva narrow, 7 mm. long, splitting at top, dingy white.

Spores smooth, ellipsoid, 3.7–4 x 6.3–7μ. Basidia 4-spored, 6.5–7.4μ thick. Cystidia bottle-shaped with a distinct neck or some without a neck, 7.4–11 x 25–35μ.

This differs from *V. cinerea* in the slender stem, white and far less flocculent cap and volva and in the somewhat smaller spores. The cystidia are about the same in both. The different surface of the cap is easily seen even in the dry state. From *V. pusilla* it differs in the tomentose and longer stem, long, slender volva, less fibrous cap, and the more narrowly ellipsoid spores. In the dry state the umbo is very conspicuous. Beardslee (1915) considers this species the same as *V. hypopithys* Fr., and from the description of the latter this sounds reasonable.

North Carolina. Chapel Hill. No. 3476. In humus among grass in a pasture, Aug. 21, 1919.
Asheville. Beardslee.

Volvaria cinerea Beardslee. Journ. E. M. Sci. Soc. **31:** 148. 1915.
?V. villosavolva Lloyd. Myc. Notes No. **4:** 31, fig. 2. 1899.

Plate 29 (bottom, left) and pl. 32, figs. 16, 17

Cap 1.4–3.8 cm. broad, hemispheric to plano-convex at full maturity and with or without a slight umbo, center dark gray-brown, fading toward margin to mouse

color with a tint of fawn or (No. 7123) light fleshy tan, obscurely striate; surface felted by interwoven, partially free fibers. Flesh membranous, toughish.

Gills rather close, free, ventricose and rounded at each end, 3 mm. broad, the margin barely granular.

Stem solid, pure white, nearly equal, 2–3 cm. long, 2–2.5 mm. thick, thinly or closely pubescent with minute curled fibers. Volva entirely free (as in *pusilla*) deep brown, thick, persistent, covered with a dense pure white felted tomentum which is longer and more conspicuous than that of the cap and stem; divided into 2 to 4 flaps.

Spores (of No. 5269) deep salmon, smooth, oblong-elliptic, one end often broader, 3.8–5 x 5.5–8μ. Besidia 8–8.5 x 20–25μ. Cystidia on edge and sides of gills, bottle-shaped, often irregular, the swollen part about 11–19μ thick, projecting up to 30–40μ.

A well marked and dainty little plant that we have found only in cultivated soil. It differs from *V. pusilla* in gray cap color, in pubescent stem and dark brown, pubescent volva. The smoother and more compact cap surface of *V. pusilla* is easily obvious even in dried plants. We are confident that our plants should be referred to Beardslee's species, although that was found by him on rotten wood. *Volvaria villosavolva* may be the same, but we have not been able to examine Lloyd's plants.

North Carolina. Chapel Hill. No. 1546. On good soil in a propagating box, June 18, 1915. Spores exactly as in No. 5259, 3.8–5.4 x 6.3–8.2μ. No. 5269. In a cultivated bed in the Drug Garden, July 21, 1922. No. 7060. In rich soil in the Drug Garden, Aug. 7, 1923. Spores 4.2–4.8 x 5.5–7μ. No. 7123. On earth with a blue-green alga in the Drug Garden, Aug. 12, 1923. Cap light fleshy tan. Volva dark gray-brown with white pubescence. Stem thinly pubescent.
Asheville. Beardslee. Type locality.

Volvaria Loweana (Berk.) Gill. Champ. Fr., p. 386. 1878.
Agaricus Lovelanus Berk. in Smith. Engl. Fl. **5**: 104. 1836.
(The species was named for the Rev. R. T. Lowe, but the spelling, as published, was incorrect.)

This rare and interesting European species has been found so far only in the northern states and Canada, but it is to be expected in our southern mountains. It is a parasite on *Clitocybe nebularis* (or nearly related forms), and is thought by Quélet and by Maire to be a more robust form of *Volvaria plumulosa* Lasch, which is not a parasite. Konrad and Maublanc (Icon. Sel. Fung., pl. 17, fig. 2) treat it as a subspecies of *V. hypopithys* (Fr.) Karst. See E. T. Harper (1916) for a discussion of *Loweana*, *plumulosa*, and other small species that are probably related. He says that Worthington Smith succeeded in growing *Loweana* on *Clitocybe nebularis*.

We give below Harper's description of *V. Loweana* as he found it in Minnesota.

Pileus 1–2 inches broad, ovoid or globose, becoming campanulate and expanded, obtuse, broadly umbonate, villous-silky, dry, not striate, fimbriate on the involute margin, white; flesh solid, white or with a tinge of pink; lamellae slightly ventricose, attenuated in each direction but somewhat broader in front, free, white-floccose on the edges, becoming pink and finally reddish with spores; stem about 2 inches long, 2–3 lines thick, slightly bulbous at the base, equal or

somewhat attenuated upward, solid, white, fibrillose; volva white, covering the bulbous base of the stem, free margin short, lobed; spores pink to reddish in mass, ovoid or ellipsoid, 4–5 x 6–7μ, membrane thick, vacuoles one or two; basidia club-shaped, enlarged below the apex, 7 x 30μ, contents granular; cystidia numerous especially on the edges of the lamellae, ventricose, acuminate above with a cylindrical obtuse apex, 8–15 x 45–70μ.

Parasitic on *Clitocybe nebularis*.

Illustrations: Berkeley. Outl. Brit. Fung., pl. 7, fig. 2.
Cooke. Ills. Brit. Fungi, pl. 295.
Gillet. Champ. Fr., pl. 712 (252).
Harper. Mycologia **8:** pl. 177, A, B. 1916.
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Juillard-Hartmann. Icon. Champ. Sup., pl. 74, fig. 4.
Konrad and Maublanc. Icon. Sel. Fung., pl. 17, fig. 2.
Maire. Bull. Soc. Myc. Fr. **27:** pl. 13, fig. 1. 1911.
Smith. Pap. Mich. Acad. Sci. **19:** pl. 44. 1934.
For illustrations of *V. plumulosa*, see Harper, as above, fig. C, and Maire, as above, fig. 2; also Lange, Agarics of Denmark, Pt. 7, pl. 1, fig. 1 a–c.

EXCLUDED SPECIES

Volvaria emendatior (B. & C.) Sacc. Syll. Fung. **14:** 125. 1899.

Agaricus emendatior B. & C. Ann. Mag. Nat. Hist., 3rd. ser. **4:** 288. 1859.

This plant has not been recognized since its original description, where it was cited from South Carolina (Curtis) and New England (Sprague). Curtis also included it in his *Catalogue of North Carolina Plants*. The original description is as follows.

Pileus 3 inches across, flat, with an obtuse umbo, smooth, white; margin thin, striate; stem 3 inches high, 3 inch thick, slightly incrassated above and below, very slightly arachnoid-fibrous, solid; volva forming merely a short rim; gills ventricose, remote, free and rounded behind, white, at length flesh-coloured, extending in front beyond the ragged margin of the pileus, as in *Montagnites*. Spores broadly cymbiform, $\frac{1}{2000}$ inch long. Smell disagreeable, but not strong. In the button state the pileus is areolate.

From this we strongly suspect that the plant is *V. speciosa*, as there is nothing to exclude it. Dried plants of *speciosa* often show a distinct central umbo, although the fresh plant did not. We do not consider the extension of the gills beyond the cap margin of any importance, as this might result in any delicate species after heavy rains.

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MURRILL. North American Flora **10:** 140–145. 1917.
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ULBRICH. Über einige seltene Volvaria-Arten. Not. Bot. Gart. u. Mus. Berlin-Dahlem 13, No. 116: 56–64. 1936.

EXPLANATION OF PLATE 32

Fig. 1. Volvaria bombycina. No. 1161. Spores.
Fig. 2. V. speciosa. No. 1475. Spores.
Figs. 3–5. V. Earlei. No. 13175. Habit sketch of young expanding plant, spores, cystidium and 4-spored basidium.
Fig. 6. V. Earlei. Type. Spores.
Fig. 7. V. floridana. Spores of plant from Smith Island, N. C.
Fig. 8. V. floridana. Type. Spores.
Fig. 9. V. volvacea. No. 3319. Spores.
Figs. 10, 11. V. pusilla. No. 7193. Spores and basidia (showing short sterigmata).
Figs. 12–15. V. pubescentipes. No. 3476. Habit sketch, spores, basidium, and four cystidia.
Figs. 16, 17. V. cinerea. No. 5269. Spores, basidium and three cystidia.
Figs. 3 and 12 × $\frac{3}{4}$; figs. 5, 11, 14, 15, 17 × 810; spores × 1620.

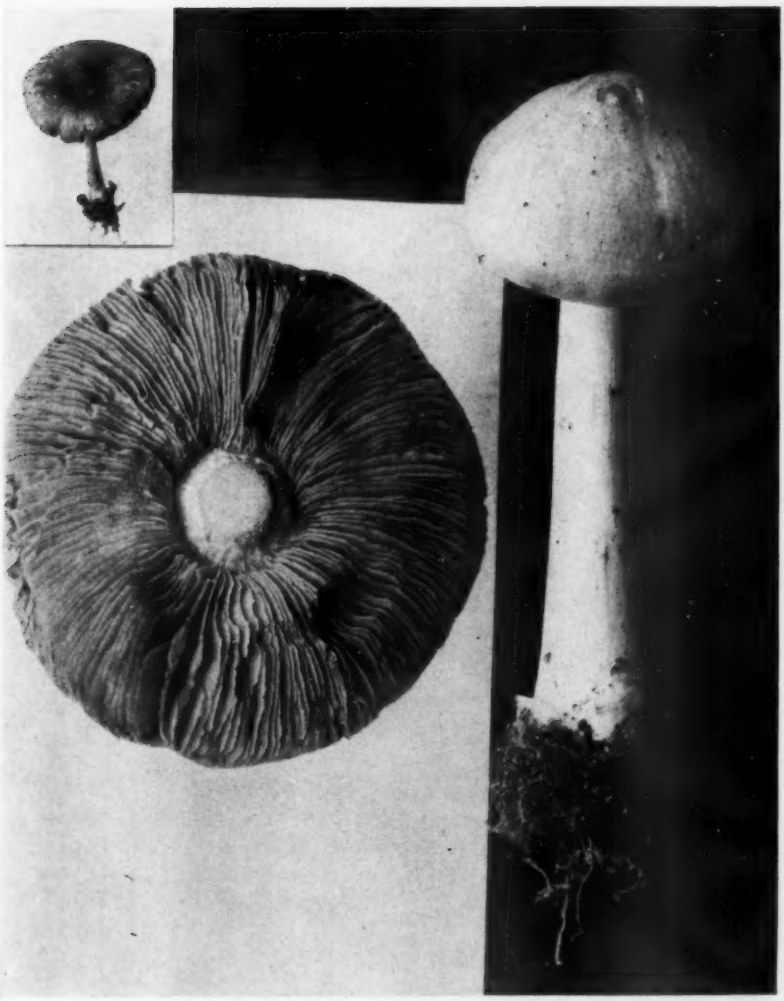
PLATE 28

Volvaria bombycina. No. 1161.

PLATE 29

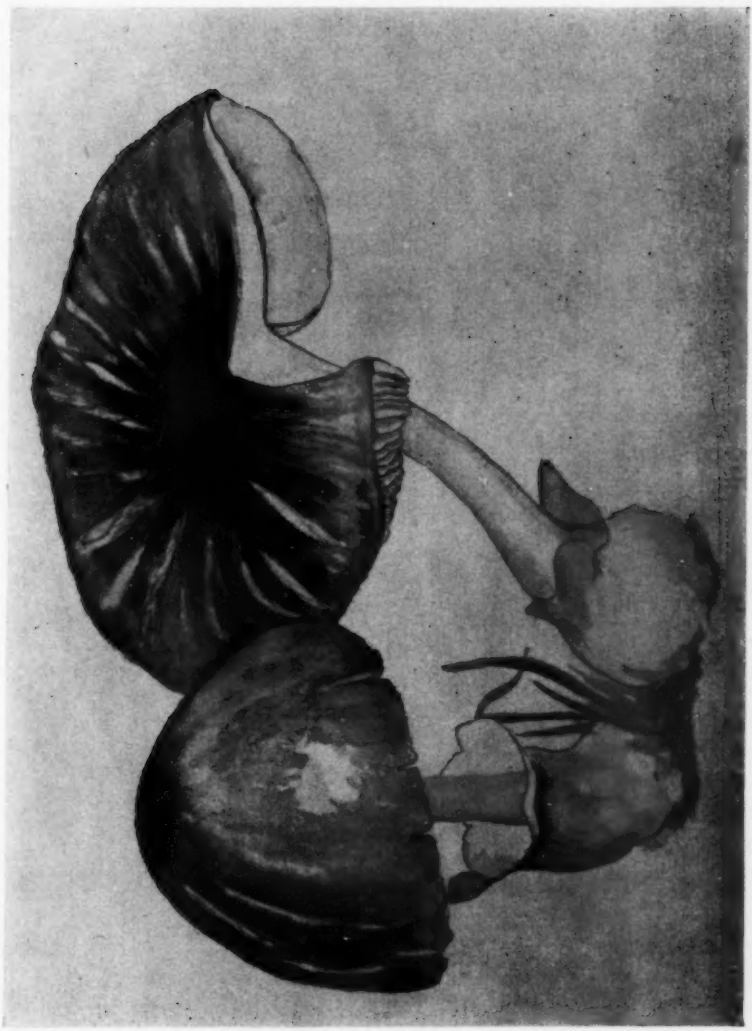
Volvaria pusilla (top). No. 7193. Nat. size.
Volvaria cinerea (left, below). No. 1546. Enlarged one third.
Volvaria Earlei (right, below). No. 13780. Nat. size.

PLATE 30



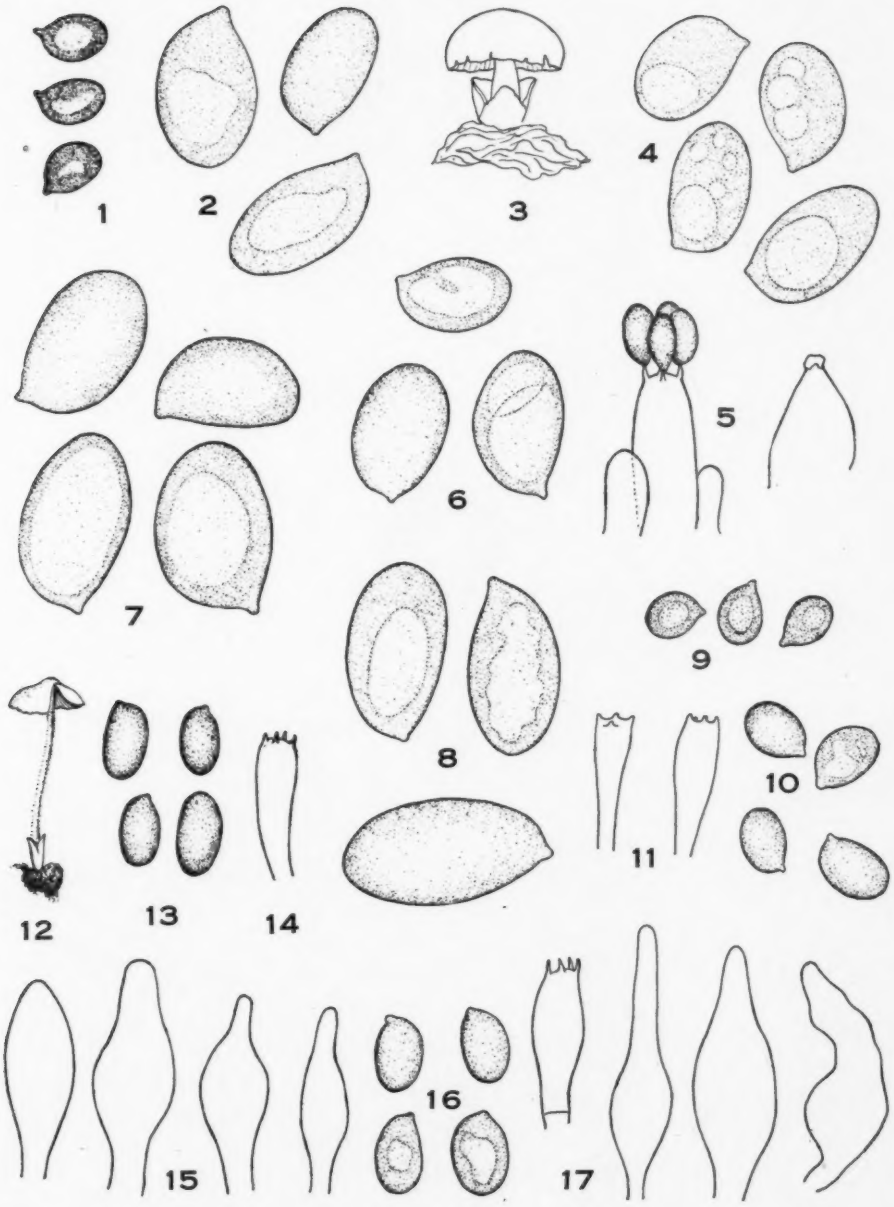
Volvaria cinerea (upper left). Asheville, N. C.
Photograph by H. C. Beardslee.
Volvaria speciosa. No. 1476. Nat. size.

PLATE 31



Volvaria volvacea. No. 3319. Nat. size.
From painting by Dorothy Coker.

PLATE 32



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BOOKS FROM CHAPEL HILL

THE BOLETACEAE OF NORTH CAROLINA. By WILLIAM CHAMBERS COKER AND ALMA HOLLAND BEERS. This study is based on notes and photographs taken by the senior author over a period of about forty years. For a good many years both authors have worked on the group and have made many collections. While most of the work has been done in Orange County and in the Blue Ridge and Alleghany Mountains of North Carolina, many specimens have been collected in South Carolina and Georgia. While the title covers only North Carolina, this work includes all except the few species reported from the neighboring states of Virginia, South Carolina, Georgia, and Alabama of which examples have not been available or that have been reduced to synonomy with species included.

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